

Title

**Summary of the Literature Review for LLCotton25 Cotton
October 1, 2021 – June 30, 2022**

Final Report

Data or guideline requirement

Explanatory note on literature searching
conducted in the context of GMO applications for (renewed) market authorization
and annual post-market environmental monitoring reports on GMOs authorised in the EU market.
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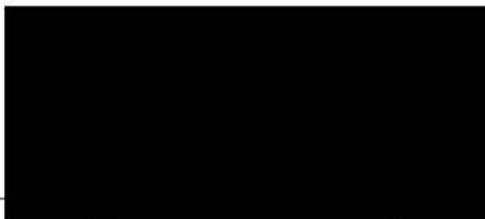
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SIGNATURE PAGE

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SUMMARY

The LLCotton25 cotton event was developed through *Agrobacterium*-mediated transformation using the vector pGSV71 containing the bar expression cassette. LLCotton25 cotton produces phosphinothricin acetyl transferase (PAT/*bar*) protein, which confers tolerance to glufosinate-ammonium herbicides. The OECD identifier is ACS-GHØØ1-3.

A scoping review was performed for the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. The objective of this scoping review was to determine if there were studies about the molecular characterization of LLCotton25 cotton, its effect on food and feed safety or environmental safety, that might require in-depth examination. A set of broad literature searches was performed using several bibliographic databases covering scientific literature from October 1, 2021 to June 30, 2022. Additional sources of information, such as web pages of food safety, agriculture, and biotechnology-related authorities were searched for the same time window, along with the bibliographies of relevant reviews. The references identified were evaluated for potential relevance to the scoping review questions according to pre-defined criteria.

These literature searches identified a total of 54 unique publications, which were subject to rapid assessment to exclude obviously irrelevant publications. One publication was progressed for detailed assessment and was determined to be not relevant after detailed review.

No new publications were found that contained new data on the molecular characterization of the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. Similarly, no new publications were found that suggested any potential adverse effects of LLCotton25 cotton on human health, animal health, or the environment. No issues or topics were identified that would trigger or warrant more specific question formulation or indicate that a systematic review would be of value.

1. INTRODUCTION

The LLCotton25 cotton event was developed through *Agrobacterium*-mediated transformation using the vector pGSV71 containing the bar expression cassette. LLCotton25 cotton produces phosphinothricin acetyl transferase (PAT/*bar*) protein, which confers tolerance to glufosinate-ammonium herbicides. The OECD identifier is ACS-GHØØ1-3.

The objective of the literature searches described here was to determine if there were studies published between October 1, 2021 and June 30, 2022 that mention the molecular characterization of LLCotton25, and/or any adverse effect of LLCotton25 in food, feed or the environment. In that context, a broad and inclusive literature search was performed, and the articles retrieved were reviewed in a comprehensive and transparent manner. This was intended as a scoping review. The literature review was performed as recommended in the European Food Safety Authority (EFSA) explanatory note on literature searching conducted in the context of Genetically Modified Organisms (GMO)¹ applications and post-market environmental monitoring activities (2019).

The literature searches were performed for the LLCotton25 and its newly expressed protein, PAT/*bar*. The search terms also included relevant synonyms, trade name and intended trait, plant species and general GMO terms. When needed, plant species and general GMO terms were used to limit the search results (described in [Section 3](#)).

2. OVERALL METHODS

2.1. Objective of the scoping review

The objective of the scoping review was to survey the evidence base for the LLCotton25 and its newly expressed protein, PAT/*bar*, in order to identify any specific issues related to food or feed safety, molecular characterization or environmental safety that might require in-depth examination.

2.2. Review questions

Review questions were formulated to conform to PE(I)CO structure (Population, Exposure (Intervention), Comparators, Outcome) if possible, and to address data requirements. They were modeled after the review question examples provided in the EFSA 2019 explanatory note¹.

Question 1: Were any studies published during the reporting period that describe adverse effects on human or animal health or the environment of the LLCotton25 and its newly expressed protein PAT/*bar*?

Key elements:

Population: Human health; animal health; environmental safety

Exposure: LLCotton25, derived food/feed products, newly expressed protein in LLCotton25

Comparators: When applicable, comparable populations or subjects exposed to appropriate controls (e.g., vehicle only, innocuous control protein, non-GM comparator) or conventional counterpart used for comparative analysis of plant material

Outcome: Adverse effects

Question 2: Were any studies published during the reporting period that focus on molecular characterization of the LLCotton25 and its newly expressed protein PAT/*bar* in crop?

Key elements:

Population: LLCotton25 and newly expressed protein in LLCotton25

Outcome: Molecular characterization (which would indicate the information/data requirement for molecular characteristics)

2.3. Criteria for relevance

Criteria for establishing the relevance of retrieved publications were defined prior to conduct of the search. These criteria were modeled after those given in the EFSA 2019 explanatory note¹ and are described in Table 1.

Table 1: Eligibility/inclusion criteria to establish the relevance of retrieved publications

| Concepts | Criteria | Comment |
|--|---|--|
| Key elements of review questions with PECO structure | | |
| Population | The publication addresses human and animal health, and/or the environment (including biodiversity, ecosystem services, service providing units, and endangered species) as general protection goals | From the publications that address the GMO under consideration, those that address protection goals relevant to the risk assessment of the GMO are eligible |
| Exposure (Intervention) | The publication addresses the GMO, derived food/feed products, and/or the intended trait(s) (e.g., newly expressed proteins(s)) that are identical or like those under regulatory review | This enables the selection of publications that address the GMO, derived food/feed products, and/or the intended trait(s) under consideration |
| Comparator | If the publication reports a comparative study that uses plant material as test material, eligible publications must report a non-GM variety as comparator | In those cases where the publication addresses the GMO under consideration, reports a comparative analysis study and uses plant material as test material, eligible publications also need to include an appropriate non-GM line as comparator |
| Outcome | The publication addresses effects/impacts on human and animal health, and/or the environment | Publications that address the GMO under consideration also need to address effects/impacts on entities of concern, and potential determinants of exposure that place these entities at risk, in order to be relevant to the risk assessment of the GMO |

| Concepts | Criteria | Comment |
|-------------------------------|---|---|
| Additional concepts | | |
| Information/data requirements | The publication reports information pertaining to one or more information/data requirement(s) outlined in Appendix A for the GMO and derived food/feed products under consideration, including the intended trait(s) | Publications that potentially contribute to the knowledge informing the risk assessment of the GMO under consideration, and thus the risk hypotheses addressed, taking account of both hazard and exposure, can be considered relevant according to this eligibility/inclusion criterion. Publications addressing other issues such as benefits, socio-economics, ethics, crop protection, detection methods, efficacy, public perception and risk communication can be excluded, as they are not necessarily relevant to the risk assessment of GMOs |
| Plant species | The publication addresses the same plant species as the GMO under consideration | This eligibility/inclusion criterion permits the exclusion of publications on GMOs that contain the same intended trait(s) as the GMO under consideration, but which are introduced in another plant species |
| Scope of GMO application | The publication addresses pathways and levels of exposure to the GMO, derived food/feed products, and the intended trait(s) that are relevant for the intended uses of the GMO and derived food/feed products under regulatory review | From the publications that address the GMO under consideration, those that consider pathways and levels of exposure relevant to the scope of the GMO application (i.e., import and processing for food/feed uses, cultivation) are eligible |
| Target pests/organisms | The publication addresses target pests/organisms that are established in the EU | This permits the exclusion of publications that address interactions between the GMO and target pests/organisms that do not occur in the EU |

| Concepts | Criteria | Comment |
|---|---|--|
| Stacked events obtained by conventional crosses/ subcombinations | The publication addresses the stacked event and not any subcombinations or the single events | This permits the selection of publications on the stacked event and the exclusion of publications on any subcombinations and the single events of the stacked event, because the risk assessment of GMO applications for stacked events covers only the products in the scope of the GMO application |
| Molecular stacks | The publication addresses: the molecular stack; all newly expressed proteins in the molecular stack; and/or one or several of the newly expressed proteins in the molecular stack that has/have not been previously risk assessed by EFSA and/or its GMO Panel and for which no safe use has been determined yet by EFSA and/or its GMO Panel | This permits the exclusion of publications that address one or several (not all) of the newly expressed proteins in the molecular stack that has/have been previously risk assessed by EFSA and/or its GMO Panel and for which the safe use has been determined by EFSA and/or its GMO Panel |
| Previously risk assessed publications | The publication has not been previously risk assessed by EFSA and/or its GMO Panel and is not cited/referenced in an EFSA/GMO Panel output | This permits the exclusion of publications that have been previously risk assessed by EFSA and/or its GMO Panel and cited/referenced in an EFSA/GMO Panel output |
| Access | Full-text document is accessible | If potentially relevant full-text documents cannot be obtained, they should be listed in a table with a description of the (unsuccessful) methods that have been used to try to obtain a copy |
| Reporting format | The publication presents original/primary data, or it is a risk assessment from a relevant key organisation (such as regulatory agencies and risk assessment bodies involved in the risk assessment of GMOs) | This permits the exclusion of publications that do not present original/primary data (e.g., editorials, position papers), and the inclusion of relevant risk assessments performed and reported by relevant key organisations. Reviews should only be included if they present data that are not available from a primary research study |

| Concepts | Criteria | Comment |
|------------------|--|---|
| Reporting format | A study in a publication should only be presented once, but if it is presented in more than one publication, all publications should be listed and grouped | Duplicate publications should be excluded at the screening stage. Only one copy of a study is required even if it is reported in different publications, and identified in more than one database |

Table adapted from 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.

2.4. Reference publication

A publication that is relevant for LLCotton25 cotton was known before starting the search, and was used as reference to test the search profile:

- Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I.C., Epstein, M.M., Firbank, L.G., Guerche, P., Hejatko, J., Moreno, F.J., Mullins, E., Nogué, F., Rostoks, N., Sánchez Serrano, J.J., Savoini, G., Veromann, E., Veronesi, F., Álvarez, F., Ardizzone, M., Paraskevopoulos, K. (2018). Assessment of genetically modified LLCotton25 for renewal of authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-010). *EFSA Journal* 16(11) e05473

Since this reference was published in 2018 (before the current search period), the search profile was tested without limiting it to documents updated during the search period (UP>=20211001 and UP<=20220630).

3. SEARCH METHODS AND OUTCOMES

The search strategies used here followed the 2019 EFSA explanatory note on literature searching conducted in the context of GMO applications and post-market environmental monitoring activities¹. The search strategies were designed to be broad and sensitive enough to capture any relevant publications, if available.

An information specialist with background in plant biotechnology selected the databases, identified relevant search terms, developed search profiles, designed search strategies, and conducted the searches.

3.1. Time window and date of the literature search

The database searches were performed on July 4, 2022. Only documents updated between October 1, 2021 and June 30, 2022, were considered in the search. The dates of most recent database updates are provided in [Table 3](#).

3.2. Databases used in the literature search

All searches were performed in the host STN (Scientific and Technical Information Network), an online database service operated jointly by CAS and FIZ Karlsruhe. STN provides access to a broad range of databases from the most renowned database producers worldwide.

The searches described here were performed in five databases: three multidisciplinary/large databases (Biosis, Medline and CA-Plus) and two subject-specific databases focused on agriculture-related topics (Agricola and CABA).

See [Appendix 1](#) for detailed database descriptions.

3.3. Search strategy

The search profile was designed to cover event name, trade name, newly expressed protein, and intended trait. Since the 'newly expressed protein' profiles and the 'intended trait' profiles produced too many results when used on their own, they were combined with additional profiles: the 'newly expressed protein' profile were combined with a 'plant species' profile while the 'intended trait' profiles were combined with a 'general GMO' profile as well as with the 'plant species' profile. The reference publication ([Section 2.4](#)) was identified by the search profiles confirming the validity of the applied search strategy. See Table 2 for a detailed search profile.

Table 2: Search profile for database search

| Set | Search string | Concepts |
|-----|--|---|
| 1 | s LLCotton25 or LLCotton(w)25 or LL(w)cotton25 or LL(w)cotton(w)25 or ACS-GH001-3 or ACS(w)GH001(w)3 or ACSGH001(w)3 or ACS-GHOO1-3 or ACS(w)GHOO1(w)3 or ACSGHOO1(w)3 | Event name |
| 2 | libertylink or libertylinktm or libertylinkrtm or liberty(w)link or liberty(w)linktm or liberty(w)linkrtm or LL or LLTM or LLRTM | Trade name |
| 3 | ((bar or pat)(2a)(gene# or protein# or enzyme#) or ppt(2w)acetyltransferase or ppt(2w)acetyl(w)transferase or pt(w)n(2w)acetyltransferase or pt(w)n(2w)acetyl(w)transferase or phosphinothricin(w)n(w)acetyltransferase or phosphinothricin(2w)acetyltransferase or phosphinothricin(2w)acetyl(w)transferase or phosphinothricinacetyl(w)transferase | Newly expressed protein |
| 4 | (herbicide? or bialaphos or basta or glufosinate or gluphosinate or phosphinothricin or liberty?)(5a)(resist? OR protect? OR toleran?) | Intended trait |
| 5 | cotton# or gossypium or G(w)hirsutum or g(w)barbadense | Plant species |
| 6 | GMO OR GMOs OR LMO OR LMOs OR GM OR GE OR transgen? OR (genetic?(3a)(modif? OR transform? OR manipulat? OR improv? OR engineer?)) | GMO general |
| 7 | (2 or 3) and 5 | (Trade name or Newly expressed protein) AND Plant species |
| 8 | 4 and 5 and 6 | Intended trait AND Plant species AND GMO general |

| Set | Search string | Concepts |
|-----|---------------|---|
| 9 | 1 or 7 or 9 | Event name OR ((Trade name OR Newly expressed protein) AND Plant species) OR (Intended trait AND Plant species AND GMO general) |

All searches were performed in the Basic Index (BI) field, which includes the following subject headings/field names:

- **Agricola:** title (TI), controlled term (CT), supplementary term (ST), abstract (AB), named person (NA), corporate name (CO), note (NTE), geographic term, CABA and other fields (GT)
- **Biosis:** title (TI), abstract (AB), biosystematic codes (BC), chemical name (CN), controlled term (CT), gene name (GEN), geographic term (GT), organism (ORGN) and supplementary term (ST); as well as CAS Registry Numbers (RN)
- **CA-Plus:** title (TI), supplementary term (ST), index term (IT) and abstract (AB); as well as CAS Registry Numbers
- **CABA:** title (TI), controlled term (CT), supplementary term (ST), broader term (BT), abstract (AB), organism name (ORGN) and geographic term (GT); as well as CAS Registry Numbers
- **Medline:** title (TI), chemical name (CN), gene name (GEN), controlled term (excluding MeSH numbers) (CT), supplementary term (ST), named person (NA), other source (OS), and abstract (AB), as well as CAS Registry Numbers and GenBank Numbers

Relevant controlled terms (Table 3) were not searched separately because they are included in the Basic Index and were captured by the free-text searches.

Table 3: Relevant controlled terms (CT) and index terms (IT) in each database

| Database | Event | New proteins | Intended traits | Plant species | GM plants |
|-----------------|-------|--------------|-----------------------------------|--|---------------------|
| Agricola | None | None | "HERBICIDE RESISTANCE" | "GOSSYPIUM BARBADENSE" "GOSSYPIUM HIRSUTUM" | "TRANSGENIC PLANTS" |
| Biosis | None | None | No terms for herbicide resistance | COTTON | None |
| CABA | None | None | "HERBICIDE RESISTANCE" | "GOSSYPIUM BARBADENSE" "GOSSYPIUM HIRSUTUM" | "TRANSGENIC PLANTS" |

| Database | Event | New proteins | Intended traits | Plant species | GM plants |
|----------|-------|--------------|------------------------|--|--------------------------------|
| CAS | None | None | "HERBICIDE RESISTANCE" | "GOSSYPIUM BARBADENSE"/CT "GOSSYPIUM HIRSUTUM"/CT | "GENETICALLY MODIFIED PLANTS" |
| Medline | None | None | "HERBICIDE RESISTANCE" | GOSSYPIUM/CT | "PLANTS, GENETICALLY MODIFIED" |

The search results were limited to documents updated between October 1, 2021 and June 30, 2022 (UP>=20211001 and UP<=20220630), and to non-patent documents (not P/DT). To ensure that documents with indexing errors where two document types (DTs) (one eligible and one ineligible) were attached to a single record were not missed, documents with both 'journal' and 'patent' as document type were also kept. These putative documents would be identified with (P/DT AND J/DT) in CABA and CAPlus.

Table 4 summarizes the number of results obtained from each of the databases searched.

See [Appendix 2](#) for a complete search history.

Table 4: Overview of the selected databases and summary of search results from each database

| Database | AGRICOLA | BIOSIS | CAB Abstracts | CAPLUS | MEDLINE |
|---|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Database Provider | STN International |
| Coverage | 1970-present | 1926-present | 1973-present | 1907-present | 1946-present |
| Date of search | 4 July 2022 |
| Datespan of the search | 1 Oct 2021 – 30 Jun 2022 |
| Latest database update | 13 June 2022 | 29 June 2022 | 29 June 2022 | 3 July 2022 | 3 July 2022 |
| Number of records retrieved | 6 | 17 | 20 | 19 | 7 |
| Number of records after duplicate removal | 4 | 15 | 15 | 13 | 7 |
| Number of relevant records after rapid assessment | 0 | 1 | 0 | 0 | 0 |

4. INTERNET and MANUAL SEARCHES

4.1. Internet Searches of food safety, agriculture, and biotechnology-related authority webpages

A search of the web pages of food safety, agriculture, and biotechnology-related authorities was conducted. Search results were manually examined for relevant records that were either published during the time period under consideration (date span of search: October 1, 2021 to June 30, 2022) or refer to relevant records published during this time frame. Relevance of results were determined based on the criteria listed in [Table 1](#) and they were summarized in [Table 5](#). All web pages searched were justified by their recommendation in the EFSA 2019 explanatory note¹. Of the 13 key organisations cited in the EFSA 2019 explanatory note¹, Environment and Climate Change Canada and Intersecretarial Commission on Biosafety of GMOs (CIBIOGEM) were excluded, since they are not involved in the risk assessment of GM plants. The US-EPA website was excluded, since the LLCotton25 does not contain an insect-resistant trait. Therefore, the internet search was limited to 10 key organisations relevant for LLCotton25 cotton. Search terms consisted of LLCotton25 or OECD Identifier or trait-specific protein(s) in LLCotton25 (all searched singly, with no search limits applied).

Table 5: Results of search of food safety, agriculture, and biotechnology-related authority websites

| Source Site Name | Website URL | Date of Most Recent Site Update | Date of Search | No. of Relevant Records |
|--|---|---------------------------------|---------------------|-------------------------|
| US Department of Agriculture (USDA) | https://www.usda.gov/ | Sep 5 2022 | Sep 5 2022 | 0 |
| US Food and Drug Administration (FDA) | https://www.fda.gov/ | Sep 7 2022 | Sep 7 2022 | 0 |
| Health Canada | https://www.canada.ca/en/health-canada.html | Aug 1 2022 | Aug 31 - Sep 1 2022 | 0 |
| Canadian Food Inspection Agency (CFIA) | https://www.canada.ca/en/food-inspection-agency.html | Aug 1 2022 | Sep 2 - 7 2022 | 0 |
| Food Standards Australia New Zealand (FSANZ) | http://www.foodstandards.gov.au/Pages/default.aspx | Aug 12 2022 | Aug 24 2024 | 0 |
| Office of the Gene Technology Regulator (OGTR) Australia | http://www.ogtr.gov.au/ | Aug 1 2022 | Aug 29 2022 | 0 |
| National Technical Commission on Biosafety (CTNBio) Brazil | http://ctnbio.mcti.gov.br/en | Aug 1 2022 | Aug 29 2022 | 0 |
| National Advisory Commission on Agricultural Biotechnology (CONABIA) Argentina | https://www.argentina.gob.ar/agroindustria/bioeconomia/biotechnologia | Sep 8 2022 | Sep 8 2022 | 0 |

| Source Site Name | Website URL | Date of Most Recent Site Update | Date of Search | No. of Relevant Records |
|--|---|---------------------------------|----------------|-------------------------|
| Genetic Engineering Approval Committee (GEAC) India | http://moef.gov.in/ | Aug 12 2022 | Aug 24 2022 | 0 |
| Ministry of Agriculture, Forestry and Fisheries (MAFF) Japan | http://www.maff.go.jp/ | Sep 6 2022 | Sep 6 2022 | 0 |

4.2. Manual searches of reference lists of recent review articles

Recent review articles as sources of reference lists to search for potentially relevant studies were identified via searches of PubMed.gov for general terms such as “GMO” or “GM crops” in the titles and abstracts. The search of PubMed.gov was also restricted to recent reviews published between October 1, 2021 and June 30, 2022. The resulting number of relevant studies found within the bibliographies of these review articles is given in Table 6.

Table 6: Documents for which reference lists were scanned for relevant studies

| No | Author(s) and Year | Title | Source | Number of relevant bibliographic references retrieved |
|----|---|---|--|---|
| 1 | Ahmad A, Munawar N, Khan Z, Qusmani AT, Khan SH, Jamil A, Ashraf S, Ghouri MZ, Aslam S, Mubarik MS, Munir A, Sultan Q, Abd-Elsalam KA, Qari SH. 2021 | An Outlook on Global Regulatory Landscape for Genome-Edited Crops | Int J Mol Sci. 2021 Oct 29;22(21):11753. | 0 |
| 2 | Halder K, Chaudhuri A, Abdin MZ, Majee M, Datta A. 2022 | RNA Interference for Improving Disease Resistance in Plants and Its Relevance in This Clustered Regularly Interspaced Short Palindromic Repeats-Dominated Era in Terms of dsRNA-Based Biopesticides | Front Plant Sci. 2022 May 13;13:885128. | 0 |
| 3 | Kawall K. 2021 | The Generic Risks and the Potential of SDN-1 Applications in Crop Plants | Plants (Basel). 2021 Oct 22;10(11):2259. | 0 |

| No | Author(s) and Year | Title | Source | Number of relevant bibliographic references retrieved |
|----|--|--|--|---|
| 4 | Lafiandra D, Sestili F, Sissons M, Kiszonas A, Morris CF. 2022 | Increasing the Versatility of Durum Wheat through Modifications of Protein and Starch Composition and Grain Hardness | Foods. 2022 May 24;11(11):1532. | 0 |
| 5 | Nagamine A, Ezura H. 2022 | Genome Editing for Improving Crop Nutrition | Front Genome Ed. 2022 Feb 9;4:850104. | 0 |
| 6 | Niraula PM, Fondong VN. 2021 | Development and Adoption of Genetically Engineered Plants for Virus Resistance: Advances, Opportunities and Challenges | Plants (Basel). 2021 Oct 29;10(11):2339. | 0 |
| 7 | Okoli AS, Blix T, Myhr AI, Xu W, Xu X. 2022 | Sustainable use of CRISPR/Cas in fish aquaculture: the biosafety perspective | Transgenic Res. 2022 Feb;31(1):1-21. | 0 |
| 8 | Then C. 2022 | Deficiencies in the Risk Assessment of Genetically Engineered Bt Cowpea Approved for Cultivation in Nigeria: A Critical Review | Plants (Basel). 2022 Jan 29;11(3):380. | 0 |
| 9 | Van Vu T. 2022 | Genome editing and beyond: what does it mean for the future of plant breeding? | Planta. 2022 May 19;255(6):130. | 0 |

5. RESULTS OF THE STUDY IDENTIFICATION AND SELECTION PROCESS

The database searches ([Section 3](#)) identified a total of 69 references, which were reduced to 54 after removal of duplicates ([Table 4](#)). No additional studies were identified in the manual searches ([Section 4](#)).

5.1. Screening of titles and abstracts to exclude obviously irrelevant references (Stage 1)

All references identified in the database searches described in [Section 3](#) were assessed for relevance based on information in their title and abstract by two reviewers independently. If opinions of relevance differed, the discrepancies were discussed between the reviewers and if a disagreement persisted, the publication under the discussion was transferred to Stage 2 for detailed evaluation by the experts. In this search, both evaluator were in 100 % agreement.

Clearly irrelevant records were tagged as “Not Relevant”. These included:

- Duplicated entries
- Secondary literature (reviews), other than assessments from regulatory agencies

- Articles on non-relevant topics like detection methods, socio-economic implications of GM crops, GM policy, agronomical performance, other herbicide resistant GM crops, other insect resistant GM crops, unrelated topics, etc.

Publications which appeared to be relevant and those of unclear relevance were tagged as “Relevant” and progressed to Stage 2 (detailed assessment; see [Section 5.2](#)).

The number of publications excluded after rapid assessment for relevance is presented in Table 7 documenting the selection process.

5.2. Detailed assessment of eligible references (Stage 2)

Publications tagged as “Relevant” in Stage 1 were assessed in detail independently by two scientific experts in each of three corresponding areas (i.e., Molecular Biology, Food and Feed Safety, Environmental Safety), based on the full text of the publications. If opinions of relevance differed between reviewers within each area, the initial reviewers discussed the discrepancy as necessary and consulted additional reviewers to resolve the discrepancy if needed.

In the relevance assessment of the literature review for the LLCotton25 cotton, reviewers agreed in 100% of the Stage 2 evaluations.

Table 7 gives an overview of the reference selection process and results of the detailed assessment.

Table 7: Results of the publication selection process

| | |
|---|----|
| Total number of publications retrieved after all searches of the scientific literature (excluding duplicates) | 54 |
| Number of publications excluded from the search results after rapid assessment for relevance (Stage 1) | 53 |
| Total number of full-text documents assessed in detail | 1 |
| Number of publications excluded from further consideration after detailed assessment for relevance (Stage 2) | 1 |
| Total number of unobtainable/unclear publications | 0 |
| Total number of relevant publications | 0 |

[Table 8](#) lists the publications determined to be relevant based on detailed evaluation. Publications that were clearly not relevant after a detailed assessment are listed in [Table 9](#). [Table 10](#) lists the publications for which full-text documents were unobtainable for detailed assessment or for which relevance was unclear after detailed assessment.

Table 8: Report of all relevant publications retrieved after detailed assessment of full-text documents for relevance: ordered by category of information/data requirement(s)

| Main category of information/data requirement | Study (Author(s) and year) | Title | Source |
|---|----------------------------|-------|--------|
| No publications in any category. | | | |

Table 9: Report of publications excluded from the risk assessment after detailed assessment of full-text documents

| Study (Author(s) and year) | Title | Source | Reason(s) for exclusion based on eligibility/inclusion criteria listed in Table 1 |
|--|--|--|---|
| Fast Brandon J. Shan Guomin Herman Rod A. Gampala Satyalinga Srinivas. 2019 | Transgene expression in sprayed and non-sprayed herbicide - tolerant genetically engineered crops is equivalent. | Regulatory toxicology and pharmacology : RTP, (2020 Mar) Vol. 111, pp. 104572. Electronic Publication Date: 26 Dec 2019 Journal code: 8214983. E-ISSN: 1096-0295. L-ISSN: 0273-2300. | The LLCotton25 cotton event was not included in this study. |

Table 10: Report of unobtainable/unclear publications

| Study (Author(s) and year) | Title | Source | Description of (unsuccessful) methods used to try and obtain a copy of the publication |
|-----------------------------------|-------|--------|--|
| No publications in this category. | | | |

6. NARRATIVE SYNTHESIS/SUMMARY OF RELEVANT STUDIES

One publication was selected during Stage 1 evaluation (rapid assessment based on title and abstract). After Stage 2 evaluation (detailed review based on full text), it was determined that the publication was not relevant for the safety assessment of the LLCotton25 and its newly expressed protein PAT/*bar*.

7. CONCLUSION

The literature searches performed for the LLCotton25 cotton and its newly expressed protein, PAT/*bar*, for the period from October 1, 2021 to June 30, 2022, identified a total of 54 unique publications (after duplicate removal). One publication was progressed for detailed assessment after excluding 53 obviously irrelevant publications during Stage 1 evaluation (rapid assessment based on title and abstract). The publication that progressed to Stage 2 was evaluated in detail, based on full text, for potential relevance, following the pre-established criteria listed in [Table 1](#).

No publications were found that contained new data on the molecular characterization of the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. Similarly, no new publications were found that suggested any potential adverse effects of LLCotton25 cotton on human health, animal health, or the environment. No issues or topics were identified that would trigger or warrant more specific question formulation or indicate that a systematic review would be of value.

8. REFERENCES

| No. | Author(s), title, source, edition, year, pages |
|-----|--|
|-----|--|

- | | |
|----|---|
| 1. | Devos Y, Guajardo IM, Alvarez F and Glanville J. 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 publications 2019:EN-1614. 62 pages. doi:10.2903/sp.efsa.2019.EN-1614. |
|----|---|

9. APPENDICES

Appendix 1 Database descriptions

| Host | File | Description |
|------|---------------|--|
| STN | AGRICOLA | <p>Agriculture Online Access is a bibliographic database containing selected worldwide literature of agriculture and related fields. AGRICOLA is the locator and bibliographic access and control system of the National Agricultural Library (NAL) collections and also includes records from other cooperating institutions. Coverage of the database includes agricultural economics and rural sociology, agricultural production, animal sciences, chemistry, entomology, food and human nutrition, forestry, natural resources, pesticides, plant science, soils and fertilizers, and water resources. Also covered are related areas such as biology and biotechnology, botany, ecology, and natural history.</p> <p>The database draws on bibliographies, serial articles, book chapters, monographs, computer files, serials, maps, audiovisuals, and reports. Bibliographic information, abstracts, geographic terms, controlled terms, and supplementary terms are searchable.</p> |
| STN | BIOSIS | <p>BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology.</p> <p>Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion.</p> <p>Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.</p> |
| STN | CABA/CAB | <p>The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including Agriculture, Agricultural chemicals, Animal sciences and production, Crop protection, Crop sciences and production, Environment, Soils and fertilizers.</p> <p>Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents.</p> <p>Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.</p> |
| STN | CAS-CA/CAPLUS | <p>The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences.</p> <p>Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered.</p> <p>Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.</p> |

| Host | File | Description |
|-------------|-------------|--|
| STN | MEDLINE | <p>MEDLINE contains information on every area of medicine. The MEDLINE database corresponds to Index Medicus, Index to Dental Literature, and International Nursing Index; OLDMEDLINE, with data from NLM's from the Cumulated Index Medicus (1960-1965) and Current List of Medical Literature (1958-1959); and, since August 2001, IN-PROCESS records, the latest documents before they have been completely indexed for inclusion on MEDLINE.</p> <p>Sources include journals and chapters in books or symposia. Bibliographic information, indexing terms, abstracts, chemical names, and CAS Registry Numbers are all searchable.</p> <p>Online thesauri are available for the Medical Subject Headings (/MN), Controlled Terms (/CT) and Chemical Name (/CN) fields.</p> |

Appendix 2 Search history

FILE 'MEDLINE' ENTERED AT 11:57:50 ON 04 JUL 2022

L1 3 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L2 14542 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L3 1511 SEA ((BAR OR PAT)(2A)(GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE

L4 204 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE

L5 1585 SEA (L3 OR L4)

L6 3563 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?)(5A)(RESIST? OR
PROTECT? OR TOLERAN?)

L7 28408 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE

L8 4007467 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A)(MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))

L9 26 SEA (L2 OR L5) AND L7

L10 114 SEA L6 AND L7 AND L8

L11 133 SEA L1 OR L9 OR L10

L12 18 SEA L11 AND PY>=2020

L13 7 SEA L12 AND UP>=20211001 AND UP<=20220630

FILE 'BIOSIS' ENTERED AT 11:58:16 ON 04 JUL 2022

L14 4 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L15 15176 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L16 2898 SEA ((BAR OR PAT)(2A)(GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE

L17 334 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE

L18 3002 SEA (L16 OR L17)

L19 10233 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?)(5A)(RESIST? OR
PROTECT? OR TOLERAN?)

L20 77053 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE

L21 478126 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A)(MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))

L22 87 SEA (L15 OR L18) AND L20

L23 261 SEA L19 AND L20 AND L21

L24 335 SEA L14 OR L22 OR L23

L25 31 SEA L24 AND PY>=2020

L26 17 SEA L25 AND UP>=20211001 AND UP<=20220630

FILE 'AGRICOLA' ENTERED AT 11:58:36 ON 04 JUL 2022

L27 2 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L28 3521 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L29 811 SEA ((BAR OR PAT)(2A)(GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L30 255 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE
L31 892 SEA (L29 OR L30)
L32 8633 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?)(5A)(RESIST? OR
PROTECT? OR TOLERAN?)
L33 66456 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L34 109651 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A)(MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))
L35 52 SEA (L28 OR L31) AND L33
L36 230 SEA L32 AND L33 AND L34
L37 272 SEA L27 OR L35 OR L36
L38 14 SEA L37 AND PY>=2020
L39 6 SEA L38 AND UP>=20211001 AND UP<=20220630

FILE 'CABA' ENTERED AT 11:59:07 ON 04 JUL 2022

L40 5 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3
L41 5659 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM
L42 1579 SEA ((BAR OR PAT)(2A)(GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L43 380 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE
L44 1681 SEA (L42 OR L43)
L45 18990 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?)(5A)(RESIST? OR
PROTECT? OR TOLERAN?)
L46 99123 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L47 193044 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A)(MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))
L48 97 SEA (L41 OR L44) AND L46
L49 513 SEA L45 AND L46 AND L47
L50 582 SEA L40 OR L48 OR L49
L51 51 SEA L50 AND PY>=2020
L52 20 SEA L51 AND UP>=20211001 AND UP<=20220630
L53 20 SEA L52 NOT P/DT
L54 0 SEA L52 AND (P/DT AND J/DT)
L55 20 SEA L53 OR L54

FILE 'HCAPLUS' ENTERED AT 11:59:40 ON 04 JUL 2022

L56 11 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3
L57 20944 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM
L58 5389 SEA ((BAR OR PAT)(2A)(GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L59 793 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE



We create chemistry

L60 5695 SEA (L58 OR L59)
L61 29118 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?)(5A)(RESIST? OR
PROTECT? OR TOLERAN?)
L62 278542 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L63 715850 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A)(MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))
L64 291 SEA (L57 OR L60) AND L62
L65 1332 SEA L61 AND L62 AND L63
L66 1514 SEA L56 OR L64 OR L65
L67 386 SEA L66 AND PY>=2020
L68 44 SEA L67 AND UP>=20211001 AND UP<=20220630
L69 19 SEA L68 NOT P/DT
L70 0 SEA L68 AND (P/DT OAND J/DT)
L71 19 SEA L69 OR L70

FILE 'MEDLINE, BIOSIS, AGRICOLA, CABA, HCAPLUS' ENTERED AT 06:00:05 ON 04
JUL 2022

L72 54 DUP REM L13 L26 L39 L55 L71 (15 DUPLICATES REMOVED)
ANSWERS '1-7' FROM FILE MEDLINE
ANSWERS '8-22' FROM FILE BIOSIS
ANSWERS '23-26' FROM FILE AGRICOLA
ANSWERS '27-41' FROM FILE CABA
ANSWERS '42-54' FROM FILE HCAPLUS