

Title**Summary of the Literature Review for MS8 x RF3, MS8 and RF3 Canola**


July 1, 2023 – June 30, 2024

Final ReportData 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.
EFSA supporting publications 2019:EN-1614

Completion date

October 14, 2024

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BelgiumReport number
24-RSOS0256Activity ID
RSOS0256

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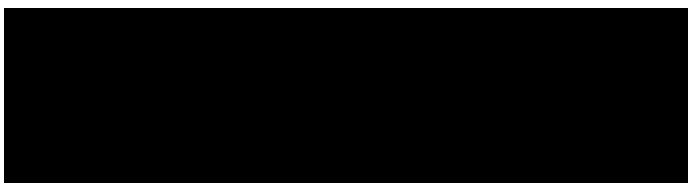
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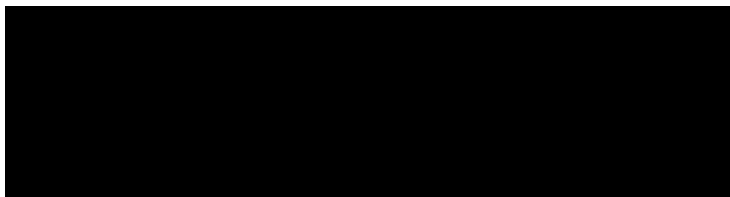
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SUMMARY

MS8 x RF3 Canola is a stacked trait product generated through conventional breeding of MS8 Canola (OECD identifier ACS-BNØØ5-8) and RF3 Canola (OECD identifier ACS-BNØØ3-6). MS8 Canola (male sterile line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW107. MS8 Canola contains the *barnase* gene (origin *Bacillus amyloliquefaciens*), coding for the Barnase protein. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetal cells during anther development. Expression of Barnase in the tapetal cells of MS8 Canola results in lack of viable pollen and male sterility. MS8 Canola also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyltransferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. RF3 Canola (fertility restorer line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW118. RF3 Canola contains the *barstar* gene (origin *Bacillus amyloliquefaciens*), coding for the Barstar protein, which is an inhibitor of the Barnase protein. The *barstar* gene is driven by the Pta29 promoter that restricts gene expression to the tapetal cells during anther development. Expression of the Barstar protein in the tapetal cells leads to restoration of fertility after crossing to a male sterile (MS) Canola line. RF3 Canola also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for PAT/*bar* conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. MS8 x RF3 Canola plants are fully fertile hybrids and express the PAT/*bar* protein which confers tolerance to glufosinate-ammonium. The OECD identifier of MS8 x RF3 Canola is ACS-BNØØ5-8 x ACS-BNØØ3-6.

A scoping review was performed for the MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins, PAT/*bar*, Barnase and Barstar. The objective of this scoping review was to determine if there were studies about the molecular characterization of MS8 x RF3, MS8 and RF3 Canola, 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 July 1, 2023 to June 30, 2024. 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 631 unique publications, which were subject to rapid assessment to exclude obviously irrelevant publications. A total of 6 publications was progressed for detailed assessment.

None of the 6 publications were determined to be relevant after detailed review. There was no new data on molecular characterization of MS8 x RF3, MS8 and RF3 Canola, or the PAT/*bar*, Barnase and Barstar proteins, nor any indications of potential adverse effects on human and animal health or on the environment. No evidence was identified that would warrant conducting a systematic review.

In summary, these literature searches and review of the retrieved articles did not identify any relevant publication that would impact the previous safety assessment of MS8 x RF3, MS8 or RF3 Canola.

1. INTRODUCTION

MS8 x RF3 Canola is a stacked trait product generated through conventional breeding of MS8 Canola (OECD identifier ACS-BNØØ5-8) and RF3 Canola (OECD identifier ACS-BNØØ3-6). MS8 Canola (male sterile line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW107. MS8 Canola contains the *barnase* gene (origin *Bacillus amyloliquefaciens*), coding for the Barnase protein. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetal cells during anther development. Expression of Barnase in the tapetal cells of MS8 Canola results in lack of viable pollen and male sterility. MS8 Canola also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyltransferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. RF3 Canola (fertility restorer line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW118. RF3 Canola contains the *barstar* gene (origin *Bacillus amyloliquefaciens*), coding for the Barstar protein, which is an inhibitor of the Barnase protein. The *barstar* gene is driven by the Pta29 promoter that restricts gene expression to the tapetal cells during anther development. Expression of the Barstar protein in the tapetal cells leads to restoration of fertility after crossing to a male sterile (MS) Canola line. RF3 Canola also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for PAT/*bar* conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. MS8 x RF3 Canola plants are fully fertile hybrids and express the PAT/*bar* protein which confers tolerance to glufosinate-ammonium. The OECD identifier of MS8 x RF3 Canola is ACS-BNØØ5-8 x ACS-BNØØ3-6.

The objective of the literature searches described here was to determine if there were publications published between July 1, 2023 and June 30, 2024 that provide information regarding the molecular characterization of the MS8 x RF3, MS8 and RF3 Canola, and/or any adverse effect of MS8 x RF3, MS8 and RF3 Canola related to 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) applications and post-market environmental monitoring activities (2019; (1)).

The literature searches were performed for the MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins, PAT/*bar*, Barnase and Barstar. The search terms also included relevant synonyms, the trade name and intended traits. When needed, plant species and general GMO terms were used to limit the search results (described in section 3.3).

2. OVERALL METHODS

2.1. Objective of the scoping review

The objective of the scoping review was to survey the evidence base for the MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins, PAT/*bar*, Barnase and Barstar, 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 note (1).

Question 1: Were any studies published during the reporting period that describe adverse effects on human or animal health or the environment of the MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins PAT/*bar*, Barnase and Barstar?

Key elements:

Population: Human health; animal health; environmental safety

Exposure: MS8 x RF3, MS8 and RF3 Canola, derived food/feed products, newly expressed proteins in MS8 x RF3, MS8 and RF3 Canola

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 MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins PAT/*bar*, Barnase and Barstar in Canola?

Key elements:

Population: MS8 x RF3, MS8 and RF3 Canola and newly expressed proteins in MS8 x RF3, MS8 and RF3 Canola

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 note (1) and are described in [Table 1](#).

The criteria listed under additional concepts were selected as appropriate to the GMO application. For example, the criteria “Stacked events obtained by conventional crosses/subcombinations” and “Molecular stacks” were not used for the relevance assessment of a “Single event” GMO search.

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

Concepts	Criteria	Comment
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
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

Concepts	Criteria	Comment
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
Stacked events obtained by conventional crosses/subcombinations	The publication addresses the higher stacked event and/or a subcombination or subcombinations of the single events of the higher stacked event, independently of its/their origin	This permits the selection of publications on the higher stacked event and/or subcombinations of the single events of the higher stacked event that are in the scope of the GMO application(s), independently of their origin. This permits the exclusion of publications on the single events of the higher stacked event, because the risk assessment of GMO applications for stacked events covers only the products in the scope of the GMO application – i.e., the higher stacked event and subcombinations of the singles involved, independently of their origin
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

Concepts	Criteria	Comment
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
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 (1).

2.4. Reference publication

Two publications related to MS8 x RF3, MS8 and RF3 Canola were previously identified and used to test and validate the search strategy:

- Naegeli, H.; Birch, A. N.; Casacuberta, J.; Schrijver, A. de; Gralak, M. A.; Guerche, P.; Jones, H.; Manachini, B.; Messean, A.; Nielsen, E. E.; Nogue, F.; Robaglia, C.; Rostoks, N.; Sweet, J.; Tebbe, C.; Visioli, F.; Wal, J. M.; Ardizzone, M.; Devos, Y.; Paraskevopoulos, K.; de Schrijver, A. (2017) Assessment of genetically modified oilseed rape MS8, RF3 and MS8 x RF3 for renewal of authorisation under regulation (EC) No 1829/2003 (application EFSA-GMO-RX-004). *EFSA Journal* 15(11):e05067;
- Zhang CJ; Yook MJ; Park HR; Lim SH; Kim JW; Nah G; Song HR; Jo BH; Roh KH; Park S; Kim DS (2018). Assessment of potential environmental risks of transgene flow in smallholder farming systems in Asia: *Brassica napus* as a case study in Korea. *The Science of the total environment* Vol 640-641, pp 688-695.

Naegeli *et al* (2017) was selected because it mentions the event names (MS8 x RF3, MS8 and RF3), the crop (oilseed rape) and one of the intended traits (herbicide tolerance). Zhang *et al* (2018) was selected because even though it is not directly relevant for MS8 x RF3 Canola or the single events, it refers to the plant species (*B. napus*) and the specific herbicide tolerance (glufosinate). Since both these articles were published before the current search period, the search profile was tested without applying the time limit used in the final search profile (UP>=20230701 and UP<=20240630).

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 activities (1). 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.

3.1. Time window and date of the literature search

The database searches were performed on July 9, 2024. Only documents updated between July 1, 2023 and June 30, 2024, were considered in the search. The dates of the most recent database updates are provided in [Table 4](#).

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 CAPlus) 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 profiles were designed to cover the event name, the trade name, the newly expressed proteins and intended traits. Since the 'newly expressed proteins' profiles and the 'intended trait' profiles produced too many results when used on their own, they were combined with additional profiles: the 'newly expressed proteins' profiles 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	MS8 or MS8x or MS-8 or MS-8x or ACS(w)BN005-8 or ACS(w)BNO05-8 or ACSBN005-8 or ACSBN005-8 or ACS(w)BN005-8x or ACS(w)BNO05-8x or ACSBN005-8x or ACSBN005-8x	Event name MS8
2	RF3 or xRF3 or RF-3 or xRF-3 or ACS(w)BN003-6 or ACS(w)BNO03-6 or ACSBN003-6 or ACSBN003-6 or	Event name RF3

Set	Search string	Concepts
	xACS(w)BN003-6 or xACS(w)BNO03-6 or xACSBNO03-6 or xACSBNO03-6 or xACSBNO03-6	
3	MS8XRF3 or MS(w)8XRF-3 or MS8(w)time#(w)RF3 or ACS(w)BN005(w)8xACS-BN003-6 or ACS(w)BNO05(w)8xACS(w)BNO03-6	Event name MS8 x RF3
4	1 or 2 or 3	Event name MS8, RF3, MS8 x RF3
5	invigor or invigorr or invigortm or in(w)vigor or in(w)vigorr or in(w)vigortm or in(w)vigorrtm	Trade name MS8, RF3, MS8 x RF3
6	barnase or RNase(w)Ba or (bacterial(w)RiboNuclease and ((Bacillus or b)(w)amyloliquefaciens)) or P00648 or IPR001887 or barstar or barnase(w)inhibitor or IPR000468 or ((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 proteins MS8, RF3, MS8 x RF3
7	(herbicid? or bialaphos or basta or glufosinate or phosphinothricin or liberty)(5a)(resist? or toleran? or protect?)or male(3a)steril? or (fertil?(3a)restor?) or restor?(w)line or pollination(w)control	Intended traits MS8, RF3, MS8 x RF3
8	((BRASSICA or B)(w)(napus or juncea)) OR RAPE? or CANOLA# OR OILSEED(w)RAPE OR oil(w)seed(w)rape or colza	Plant species
9	GMO OR GMOs OR LMO OR LMOs OR GM OR GE OR transgen? OR (genetic?(3w)(modif? OR transform? OR manipulat? OR improv? OR engineer?)) or (stacked(w)(gene# or trait# or event#))	GMO general
10	7 and 8 and 9	Intended traits MS8, RF3, MS8 x RF3 AND Plant species AND GMO general
11	4 or 5 or 6 or 10	Event name MS8, RF3, MS8 x RF3 OR Trade name MS8, RF3, MS8 x RF3 OR Newly expressed proteins MS8, RF3, MS8 x RF3 OR (Intended traits MS8, RF3, MS8 x RF3 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)
- **CAPlus:** 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" "MALE STERILITY"	"BRASSICA NAPUS" CANOLA	"TRANSGENIC PLANTS"
Biosis	None	None	No terms for herbicide resistance "MALE STERILITY"	None	None
CABA	None	None	"HERBICIDE RESISTANCE" "MALE STERILITY"	RAPE	"TRANSGENIC PLANTS"
CAPlus	None	None	"HERBICIDE RESISTANCE" No terms for male sterility	CANOLA "BRASSICA NAPUS"	"GENETICALLY MODIFIED PLANTS"
Medline	None	None	"HERBICIDE RESISTANCE" No terms for male sterility	BRASSICA NAPUS	"PLANTS, GENETICALLY MODIFIED"

The search results were limited to documents updated between July 1, 2023 and June 30, 2024 (UP>=20230701 and UP<=20240630), 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	CABA	CAPlus	Medline
Database Provider	STN International	STN International	STN International	STN International	STN International
Coverage	1970-present	1926-present	1973-present	1907-present	1946-present
Date of search	July 9, 2024	July 9, 2024	July 9, 2024	July 9, 2024	July 9, 2024
Datespan of the search	July 1, 2023 - June 30, 2024	July 1, 2023 - June 30, 2024	July 1, 2023 - June 30, 2024	July 1, 2023 - June 30, 2024	July 1, 2023 - June 30, 2024
Latest database update	Jun 11, 2024	July 5, 2024	Jun 11, 2024	July 8, 2024	July 8, 2024
Number of records retrieved	79	165	126	312	141
Number of records after duplicate removal	47	132	92	219	141
Number of relevant records after rapid assessment	1	2	0	2	1

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: July 1, 2023 and June 30, 2024) 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 note (1). Of the 13 key organisations cited in the EFSA 2019 explanatory note (1), 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 MS8, RF3

and MS8 x RF3 Canola do not contain an insect-resistant trait. The GEAC website was excluded, since this agency has only regulated GM cotton products. The CTNBio and CONABIA websites were excluded, since these agencies have not regulated any GM products for Canola. Therefore, the internet search was limited to 7 key organisations relevant for MS8 x RF3, MS8 and RF3 Canola. Search terms consisted of MS8 x RF3, MS8 and RF3 Canola, related OECD Identifiers or trait-specific proteins in MS8 x RF3, MS8 and RF3 Canola (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/	July 25, 2024	July 25, 2024	0
US Food and Drug Administration (FDA)	https://www.fda.gov/	July 29, 2024	July 29, 2024	0
Health Canada	https://www.canada.ca/en/health-canada.html	July 10, 2024	July 11, 2024	0
Canadian Food Inspection Agency (CFIA)	https://www.canada.ca/en/food-inspection-agency.html	July 10, 2024	July 12, 2024 to July 16, 2024	0
Food Standards Australia New Zealand (FSANZ)	http://www.foodstandards.gov.au/Pages/default.aspx	July 17, 2024	July 17, 2024	0
Office of the Gene Technology Regulator (OGTR) Australia	http://www.ogtr.gov.au/	July 17, 2024	July 17, 2024	0
Ministry of Agriculture, Forestry and Fisheries (MAFF) Japan	http://www.maff.go.jp/	July 17, 2024	July 17, 2024	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 July 1, 2023 and June 30, 2024. 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	Ahmar S, Hensel G, Gruszka D. 2023	CRISPR/Cas9-mediated genome editing techniques and new breeding strategies in cereals - current status, improvements, and perspectives	Biotechnol Adv. 2023 Dec;69:108248.	0
2	Chaudhury R, Chakraborty A, Rahaman F, Sarkar T, Dey S, Das M. 2024	Mycorrhization in trees: ecology, physiology, emerging technologies and beyond	Plant Biol (Stuttg). 2024 Mar;26(2):145-156.	0
3	Dolezel M, Lang A, Greiter A, Miklau M, Eckerstorfer M, Heissenberger A, Willée E, Züghart W. 2024	Challenges for the Post-Market Environmental Monitoring in the European Union Imposed by Novel Applications of Genetically Modified and Genome-Edited Organisms	BioTech (Basel). 2024 May 15;13(2):14.	0
4	Goodman RE. 2024	Twenty-eight years of GM Food and feed without harm: why not accept them?	GM Crops Food. 2024 Dec 31;15(1):40-50.	0
5	Koller F, Cieslak M. 2023	A perspective from the EU: unintended genetic changes in plants caused by NGT-their relevance for a comprehensive molecular characterisation and risk assessment	Front Bioeng Biotechnol. 2023 Oct 27;11:1276226.	0
6	Ledesma AV, Van Eenennaam AL. 2024	Global status of gene edited animals for agricultural applications	Vet J. 2024 May 22:106142.	0
7	Mat Jalaluddin NS, Ahmad Fuaad AA,	Regulatory landscape and public perception for gene-edited bananas in	Transgenic Res. 2024 Jun;33(3):89-97.	0

	Othman RY. 2024	the Southeast Asian region.		
8	Mmbando GS. 2023	The legal aspect of the current use of genetically modified organisms in Kenya, Tanzania, and Uganda.	GM Crops Food. 2023 Dec 31;14(1):1-12.	0
9	Ruiz-Zambrano NL, Pérez-Carrillo E, Serna-Saldívar SO, Tejada-Ortigoza V. 2023	Effect of thermal, nonthermal, and combined treatments on functional and nutritional properties of chickpeas	Crit Rev Food Sci Nutr. 2023 Jul 27:1-19.	0
10	Salazar-Cerezo S, de Vries RP, Garrigues S. 2023	Strategies for the Development of Industrial Fungal Producing Strains	J Fungi (Basel). 2023 Aug 8;9(8):834.	0
11	Sarker PK, Paul AS, Karmoker D. 2023	Mitigating climate change and pandemic impacts on global food security: dual sustainable agriculture approach (2S approach)	Planta. 2023 Oct 25;258(6):104.	0
12	Tanny T, Sallam M, Soda N, Nguyen NT, Alam M, Shiddiky MJA. 2023	CRISPR/Cas-Based Diagnostics in Agricultural Applications	J Agric Food Chem. 2023 Aug 9;71(31):11765-11788.	0
13	Vasudevan SN, Pooja SK, Raju TJ, Damini CS. 2023	Cisgenics and intragenics: boon or bane for crop improvement	Front Plant Sci. 2023 Nov 28;14:1275145.	0

5. RESULTS OF THE STUDY IDENTIFICATION AND SELECTION PROCESS

The database searches ([Section 3](#)) identified a total of 823 references, which were reduced to 631 references 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 discussion was transferred to Stage 2 for detailed evaluation by the experts. In this search, both evaluators 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 tolerant 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 MS8 x RF3, MS8 and RF3 Canola, 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)	631
Number of publications excluded from the search results after rapid assessment for relevance (Stage 1)	625
Total number of full-text documents assessed in detail	6
Number of publications excluded from further consideration after detailed assessment for relevance (Stage 2)	6
Total number of unobtainable/unclear publications	0
Total number of relevant publications	0

Table 8 lists the publications determined to be relevant based on the 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.

6. NARRATIVE SYNTHESIS/SUMMARY OF RELEVANT STUDIES

A total of 6 publications 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 none of these publications were relevant for the safety assessment of the MS8 x RF3, MS8 and RF3 Canola and their newly expressed proteins, PAT/*bar*, Barnase and Barstar.

Table 11 lists the relevant publications along with a summary of any adverse effects reported.

7. CONCLUSION

The literature searches performed for the MS8 x RF3, MS8 and RF3 Canola and its newly expressed proteins, PAT/*bar*, Barnase and Barstar, for the period from July 1, 2023 to June 30, 2024, identified a total of 631 unique publications (after duplicate removal). A total of 6 publications was progressed for detailed assessment after excluding 625 obviously irrelevant publications during Stage 1 evaluation (rapid assessment based on title and abstract).

The 6 publications that progressed to Stage 2 were evaluated in detail, based on full text, for potential relevance, following the pre-established criteria listed in Table 1. No relevant publications with bearing on molecular characterization, human and animal safety or environmental safety were identified. No issues or topics were identified that would trigger or warrant more specific question formulation.

8. REFERENCES

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

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 this 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
Weidner, Christopher; Koepfel, Rene; Freyer, Regina; Richl, Petra; Lieske, Kathrin; Mankertz, Joachim; Waiblinger, Hans-Ulrich (2024)	Development and validation of a multiplex real-time PCR method for screening genetically modified plants	Journal fuer Verbraucherschutz und Lebensmittelsicherheit (2024), 19(2), 165-174 CODEN: JVLOA5; ISSN: 1661-5751	The publication focuses on establishing a detection method and does not contribute to the risk assessment of MS8, RF3 or MS8 x RF3 Canola.
Travers Steven E; Bishop D Bryan; Sagers Cynthia L (2024)	Persistence of genetically engineered canola populations in the U.S. and the adventitious presence of transgenes in the environment	PloS one, (2024) Vol. 19, No. 5, pp. e0295489. Electronic Publication Date: 22 May 2024 Journal code: 101285081. E-ISSN: 1932-6203. L-ISSN: 1932-6203. Report No.: PMC-PMC11111013.	The publication is not related to MS8, RF3 or MS8 x RF3 Canola.
Tilleria, Sofia G.; Pandolfo, Claudio; Presotto, Alejandro; Ureta, Soledad (2024)	No fitness cost in <i>Brassica rapa</i> L. (wild turnip) populations associated with transgenic glyphosate and non-transgenic AHAS -inhibiting herbicides resistance	Crop Protection (2024), 177, 106527 CODEN: CRPTD6; ISSN: 0261-2194	The publication is not related to MS8, RF3 or MS8 x RF3 Canola.
Singh, Monika [Reprint Author]; Paliwal, Aparna; Kaur, Kushaldeep; Palit, Paramita; Randhawa, Gurinderjit (2023)	Development and utilization of analytical methods for rapid GM detection in processed food products: a case study for regulatory requirement	Journal of Plant Biochemistry and Biotechnology, (SEP 2023) Vol. 32, No. 3, pp. 511-524. http://www.springerlink.com/content/0971-7811 . ISSN: 0971-7811. E-ISSN: 0974-1275.	The publication focuses on establishing a detection method and does not contribute to the risk assessment of MS8, RF3 or MS8 x RF3 Canola.

Shao, Zicheng; Huang, Lei; Zhang, Yuchi; Qiang, Sheng; Song, Xiaoling [Reprint Author] (2022)	Transgene Was Silenced in Hybrids between Transgenic Herbicide -Resistant Crops and Their Wild Relatives Utilizing Alien Chromosomes	Plants-Basel, (DEC 2022) Vol. 11, No. 23, pp. Article No.: 3187. http://www.mdpi.com/journal/plants . ISSN: 2223-7747. E-ISSN: 2223-7747.	The publication is not related to MS8, RF3 or MS8 x RF3 Canola.
Wu, Wei; Ma, Bao-Luo (2022)	Understanding the trade-off between lodging resistance and seed yield, and developing some non-destructive methods for predicting crop lodging risk in canola production	Field crops research (1 Nov 2022), Volume 288, pp. 108691 ISSN: 0378-4290	The publication is not related to MS8, RF3 or MS8 x RF3 Canola.

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			

Table 11: Summary report for all relevant publications retrieved after detailed assessment of full-text documents for relevance and implications for the risk assessment: ordered by category of information/data requirement(s)

Main category of information/data requirement	Study (Author(s) and year)	Intervention/ test materials used	Adverse effects reported	Which adverse effect reported	Implications for risk assessment
No publications in this category					

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-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, BIOSIS, CABA, AGRICOLA, HCAPLUS' ENTERED AT 12:22:14 ON 09
JUL 2024
L1      1580 SEA SPE=ON  PLU=ON  MS8 OR MS8X OR MS-8 OR MS-8X OR ACS(W)
        BN005-8 OR ACS(W) BNO05-8 OR ACSBN005-8 OR ACSBNO05-8 OR
        ACS(W) BN005-8X OR ACS(W) BNO05-8X OR ACSBN005-8X OR ACSBNO05-8
        X
L2      2483 SEA SPE=ON  PLU=ON  RF3 OR XRF3 OR RF-3 OR XRF-3 OR ACS(W)
        BN003-6 OR ACS(W) BNO03-6 OR ACSBN003-6 OR ACSBNO03-6 OR
        XACS(W) BN003-6 OR XACS(W) BNO03-6 OR XACSBNA~A~3-6 OR
        XACSBNO03-6 OR XACSBNO03-6
L3      49 SEA SPE=ON  PLU=ON  MS8XRF3 OR MS(W) 8XRF-3 OR MS8(W) TIME#(W)
        RF3 OR ACS(W) BN005(W) 8XACS-BN003-6 OR ACS(W) BNO05(W)
        8XACS(W) BNO03-6
L4      3978 SEA SPE=ON  PLU=ON  L1 OR L2 OR L3
L5      1597 SEA SPE=ON  PLU=ON  INVIGOR OR INVIGORR OR INVIGORTM OR IN(W)
        VIGOR OR IN(W) VIGORR OR IN(W) VIGORTM OR IN(W) VIGORRTM
L6      3079 SEA SPE=ON  PLU=ON  BARNASE OR RNASE(W) BA OR (BACTERIAL(W)
        RIBONUCLEASE AND ((BACILLUS OR B)(W) AMYLOLIQUEFACIENS)) OR
        P00648 OR IPR001887
L7      1554 SEA SPE=ON  PLU=ON  BARSTAR OR BARNASE(W) INHIBITOR OR
        IPR000468
L8      14134 SEA SPE=ON  PLU=ON  ((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
L9      2029 SEA SPE=ON  PLU=ON  PHOSPHINOTHRICIN(W) N(W) ACETYLTRANSFERASE
        OR PHOSPHINOTHRICIN(2W) ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2
        W) ACETYL(W) TRANSFERASE OR PHOSPHINOTHRICINACETYL(W) TRANSFERA
        SE
L10     18290 SEA SPE=ON  PLU=ON  (L6 OR L7 OR L8 OR L9)
L11     77392 SEA SPE=ON  PLU=ON  (HERBICID? OR BIALAPHOS OR BASTA OR
        GLUFOSINATE OR PHOSPHINOTHRICIN OR LIBERTY)(5A)(RESIST? OR
        TOLERAN? OR PROTECT?)
L12     97688 SEA SPE=ON  PLU=ON  MALE(3A) STERIL? OR (FERTIL?(3A) RESTOR?)
        OR RESTOR?(W) LINE OR POLLINATION(W) CONTROL
L13     160866 SEA SPE=ON  PLU=ON  (L11 OR L12)
L14     312537 SEA SPE=ON  PLU=ON  ((BRASSICA OR B)(W)(NAPUS OR JUNCEA)) OR
        RAPE? OR CANOLA# OR OILSEED(W) RAPE OR OIL(W) SEED(W) RAPE OR
        COLZA
L15     5890093 SEA SPE=ON  PLU=ON  GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR
        TRANSGEN? OR (GENETIC?(3W)(MODIF? OR TRANSFORM? OR MANIPULAT?
        OR IMPROV? OR ENGINEER?)) OR (STACKED(W)(GENE# OR TRAIT# OR
        EVENT#))
L16     3548 SEA SPE=ON  PLU=ON  L13 AND L14 AND L15
L17     26945 SEA SPE=ON  PLU=ON  L4 OR L5 OR L10 OR L16
L18     2594 SEA SPE=ON  PLU=ON  L17 AND PY>=2022
L19     1002 SEA SPE=ON  PLU=ON  L18 AND UP>=20230701 AND UP<=20240630
L20     823 SEA SPE=ON  PLU=ON  L19 NOT P/DT
L21     0 SEA SPE=ON  PLU=ON  L19 AND (P/DT AND (J/DT OR A/DT))
        SET MSTEPS ON
        D SET
L22     141 SEA SPE=ON  PLU=ON  L20 OR L21
L23     165 SEA SPE=ON  PLU=ON  L20 OR L21
L24     126 SEA SPE=ON  PLU=ON  L20 OR L21
L25     79 SEA SPE=ON  PLU=ON  L20 OR L21
L26     312 SEA SPE=ON  PLU=ON  L20 OR L21
TOTAL FOR ALL FILES
L27     823 SEA SPE=ON  PLU=ON  L20 OR L21
L28     631 DUP REM L27 (192 DUPLICATES REMOVED)
        D L28 ALL TOT
```