ANSES Opinion
Request No. 2022-SA-0101
Related requests Nos. 2003-SA-0046, 2003-SA-0300, 2011-SA-0322, 2017-SA-0010, 2022-SA-0007; Nos. 2004-SA-0152, 2008-SA-0112, 2011-SA-0286, 2013-SA-0028, 2014-SA-0147, 2015-SA-0015, 2016-SA-0122, 2016-SA-0237, 2017-SA-0227; No. 2012-SA-0112

The Director General

Extract from the Opinion of 17 January 2023 of the French Agency for Food, Environmental and Occupational Health & Safety

on the assessment of management measures following the "accidental" release of genetically modified oilseed rape into the environment

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It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 17 January 2023 shall prevail.

This document is an extract from the Opinion of 17 January 2023[1], following the deletion of confidential information protected by commercial secrecy.

On 25 May 2022, ANSES received a formal request from the Directorate General for Food (DGAL) to undertake the following expert appraisal: "Request for an opinion on management measures following the 'accidental'[2] release of genetically modified oilseed rape into the environment."

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 13 April 2022, the presence of genetically modified (GM) oilseed rape was reported by the Inf'OGM association[3]. It had been found growing by the side of the road in an industrial port area near Rouen (Seine-Maritime, France). The control services of the Ministry of Agriculture and Food Sovereignty took samples on 19 and 29 April 2022, which were analysed by the

[1] Cancels and replaces the Opinion of 10 January 2023. The corrections made are described in Annex 5.
[2] Term used in this opinion to indicate unintentional release, but which does not necessarily have the one-off nature usually associated with the term "accident".
[3] Inf'OGM is an association under the French Act of 1901, which presents itself as a citizen's watchdog with regard to GMOs and seeds.

BioGEVES laboratory[4], the National Reference Laboratory[5] for the detection of genetically modified oilseed rape (seeds and vegetative parts). These analyses confirmed the presence of GM oilseed rape in the aforementioned environment.

These GM varieties of oilseed rape had been granted marketing authorisation under Regulation (EC) No 1829/2003[6] for import, processing and use in food and feed. They were also authorised as a constituent, in whole or in part, of products other than food or feed. No authorisation had been granted for their cultivation, which is therefore prohibited in the European Union (EU).

As a result, in early May 2022, the DGAL asked Haropa Port, the public establishment responsible for maintaining the industrial port area (excluding industrial sites), to destroy the oilseed rape plants by mechanical means (mowing and brush clearing). This operation was to be repeated every month, to prevent any regrowth from flowering, and producing pollen and seeds that could eventually germinate. At the same time, the DGAL also asked for monitoring to be put in place for subsequent years, to ensure the absence of any oilseed rape regrowth that could result from the germination of seeds already in the soil.

The DGAL's objective was to avoid any production and release of pollen and seeds by these GM oilseed rape plants, and to ensure their destruction, since their cultivation is prohibited.

The DGAL therefore asked ANSES to do the following:

1) Assess the suitability of the prescribed measures in relation to this objective;
2) If necessary, identify additional measures to avoid any persistence of this GM oilseed rape at the sites where it was detected; ANSES should then specify the frequency and duration of the measures to be applied;
3) Make recommendations on suitable monitoring measures (type, frequency and duration over time) to verify that the objective has been achieved.

ANSES stresses that it is responding to this request in a regulatory context in which a post-authorisation environmental monitoring plan for these varieties of GM oilseed rape has been implemented in connection with the authorisations issued over the last few years under Regulation (EC) No 1829/2003 for:

- (1) foods and food ingredients containing, consisting of or produced from these varieties of oilseed rape,
- (2) feed containing, consisting of or produced from these varieties of oilseed rape,
- (3) products consisting of or containing these varieties of oilseed rape, for any use other than those defined in points (1) and (2), with the exception of cultivation.

[4] Molecular Biology and Biochemistry Laboratory of the Study Group for the Control of Varieties and Seeds (BioGEVES).
[5] By Ministerial Order of 29 December 2009 appointing national reference laboratories in the areas of veterinary public health and plant health. French Official Journal No. 0005 of 7 January 2010, Text No. 37.
[6] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

The collective expert appraisal was carried out by the Working Group (WG) on Biotechnology, which met on 20 May, 15 June, 7 July, 3 August, 22 September, 12 October, 16 November and 14 December 2022, on the basis of initial reports written by two rapporteurs.

The expert appraisal work relating to these requests from the DGAL was preceded by a reframing of the "accidental" release of the GM oilseed rape, with all the information made available by the DGAL. It also included an update of the literature relating to the environmental risk assessment that had been carried out as part of the marketing authorisation application for these varieties of oilseed rape.

The preparatory work for the expert appraisal identified the presence of agricultural fields near the site concerned by the "accidental" release of the GM oilseed rape. Considering that Article 4.6 of Directive 2001/18/EC[7] stipulates that "Member States shall take measures to ensure traceability, in line with the requirements laid down in Annex IV, at all stages of the placing on the market of GMOs authorised under part C", ANSES proposed adding the following additional point to the expert appraisal contract sent to the DGAL:

4) In the interests of consumer information, should specific checks be carried out on products derived from crops grown near the sites where "accidental" release was detected, to ensure the absence of contamination by GM oilseed rape at a threshold of more than 0.9%, when these products are intended for food consumption?

Following the analysis of the formal request by the WG on Biotechnology, a letter requesting additional information was sent to the DGAL (Annex 2).

Lastly, as part of the work to address this formal request, a hearing was organised with the Inf'OGM association, which had initially raised the alert.

The following information was taken into account when conducting this expert appraisal:

- The formal request letter from the DGAL, specifying the context (Annex 1).
- The opinions of the Biomolecular Engineering Commission (CGB) and the High Council for Biotechnology (HCB) on the varieties of GM oilseed rape concerned by this release.
- The opinions of EFSA on the varieties of GM oilseed rape concerned by this release.
- The decisions to grant marketing authorisation under Regulation (EC) No 1829/2003 for the varieties of GM oilseed rape concerned by this release.
- The monitoring reports on environmental effects, provided for as part of the marketing authorisation procedure for the varieties of GM oilseed rape concerned by this release.

[7] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. For GMOs authorised under Regulation (EU) No 1829/2003, the general obligations set out in Directive 2001/18/EC (Part A) apply.

- The data provided by the DGAL following ANSES's request for additional information:
  - Technical instructions concerning the site inspection.
  - Site inspection records and reports.
  - BioGEVES analysis reports.
  - Internal procedures applied at the Saipol manufacturing facility in Grand-Couronne.
- Report of the hearing with the Inf'OGM association (Annex 3).
- The literature data listed in this opinion. The literature search was carried out independently by two experts of the WG on Biotechnology, following the ANSES "Guide to bibliographical research" (ANSES/PG/0128) and on the basis of the following keywords (non-exhaustive list in French and in English, cross-referenced or not in the search equations): oilseed rape, *Brassica napus*, *Brassica*, canola, release, environmental risk, hybrids, pollinators, feral populations, spontaneous populations, gene flow, pollen flow, glyphosate resistance, glufosinate-ammonium resistance, persistence, pollen dispersal by insects, seed dormancy, seed bank, seed germination, vernalisation, flowering period.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE WG ON BIOTECHNOLOGY

3.1. Regulatory background

3.1.1. Genetically modified oilseed rape authorised in the European Union under Regulation (EC) No 1829/2003

Within the European Union (EU), the GM oilseed rape authorised at the time of receipt of this formal request had been authorised under Regulation (EC) No 1829/2003 for import, processing and use as food and feed, and for all other uses except cultivation[8]. Cultivation of this GM oilseed rape is therefore prohibited.

Table 1: Summary of marketing authorisations under Regulation (EC) No 1829/2003 for GM oilseed rape authorised at the time of receipt of this formal request, and of opinions on applications for authorisation

GM oilseed rape (transformation events)	Marketing authorisation decisions (in force)	EFSA GMO Panel dossier numbers and opinions	CGB*/HCB opinions	AFSSA**/ANSES opinions
GT73	(EU) 2021/1385 of 17 August 2021 (OJ L 300, 24.8.2021, pp.4-9) (according to Regulation (EC) No 1829/2003) (EU) 2015/701 of 24 April 2015 (OJ L 112, 30.4.2015, pp. 86-90) (according to Regulation (EC) No 1829/2003)	Dossier C/NL/98/11. 2004 Opinion. Dossier EFSA-GMO-RX-GT73. 2009 Opinion. Dossier EFSA-GMO-NL-2010-87. 2013 Opinion. Dossier EFSA-GMO-RX-002. 2020 Opinion. Dossier EFSA-GMO-RX-026/1. 2022 Opinion. Dossier EFSA-GMO-RX-026/2. 2022 Opinion.	CGB (2003) – Opinion of 13 March 2003. CGB (2003) – Opinion of 30 September 2003.	AFSSA (2003) Request No. 2003-SA-046 AFSSA (2004) Request No. 2003-SA-0300 ANSES (2012) Request No. 2011-SA-0322 ANSES (2022) Request No. 2022-SA-0007
Ms8, Rf3, Ms8xRf3	(EU) 2013/327 of 25 June 2013 (OJ L 175, 27.6.2013, p. 57) (according to Regulation (EC) No 1829/2003). (EU) 2019/1301 of 26 July 2019 (OJ L 204 of 2.08.2019, pp. 50-53	Dossier EFSA-GMO-RX-MS8-RF3. 2009 Opinion. Dossier EFSA-GMO-BE-2010-81. 2012 Opinion. Dossier EFSA-GMO-RX-004. 2017 Opinion.	CGB (2004) – Opinion of 11 May 2004. CGB (2004) – Opinion of 3 December 2004.	AFSSA (2004) Request No. 2004-SA-0152 AFSSA (2004) Request No. 2004-SA-0374 AFSSA (2008) Request No. 2008-SA-0112

[8] Only one GM maize variety is permitted to be grown in the EU: MON810 maize, grown in Spain and Portugal. In France, Act No. 2014-567 of 2 June 2014 prohibits the cultivation of GM maize varieties. In addition, Commission Implementing Decision (EU) 2016/321 of 3 March 2016 adjusting the geographical scope of the authorisation for cultivation of genetically modified maize MON810, prohibits the cultivation of MON810 maize in France and in all other Member States or regions that have applied for a geographical exclusion.

	(according to Regulation (EC) No 1829/2003)			ANSES (2012) Request No. 2011-SA-0286 ANSES (2018) Request No. 2017-SA-0227
T45	(EU) 2019/2081 of 28 November 2019 (OJ L 316, 6.12.2019, p. 57) (according to Regulation (EC) No 1829/2003)	Dossiers EFSA-GMO-UK-2005-25 and EFSA-GMO-RX-T45. 2008 Opinion. Dossier EFSA-GMO-RX-012. 2019 Opinion.		AFSSA (2007) Request No. 2007-SA-0126
MON 88302	(EU) 2015/687 of 24 April 2015 (OJ L 112, 30.4.2015, p. 22) (according to Regulation (EC) No 1829/2003).	Dossier EFSA-GMO-BE-2011-101. 2014 Opinion.	HCB, CS (2012) – Opinion of 3 July 2012.	ANSES (2012) Request No. 2012-SA-0112
Ms8xRf3xGT73, Ms8xGT73, Rf3xGT73	(EU) 2021/1391 of 17 August 2021 (OJ L 300, 24.8.2021, p. 41) (according to Regulation (EC) No 1829/2003).	Dossier EFSA-GMO-NL-2009-75. 2016 and 2020 Opinions.	HCB, CS (2013) – Opinion of 3 May 2013.	ANSES (2013) Request No. 2013-SA-0028 ANSES (2016) Request No. 2016-SA-0122
73496	(EU) 2022/529 of 31 March 2022 (OJ L 105, 4.4.2022, p. 39) (according to Regulation (EC) No 1829/2003)	Dossier EFSA-GMO-NL-2012-109. 2021 Opinion.		ANSES (2013) Request No. 2012-SA-0265 ANSES (2021) Request No. 2021-SA-0124
MON 88302xMs8xRf3, MON 88302xMs8, MON 88302xRf3	(EU) 2017/2453 of 21 December 2017 (OJ L 346, 28.12.2017, p.31) (according to Regulation (EC) No 1829/2003)	Dossier EFSA GMO-NL-2013-119. 2017 Opinion.		ANSES (2014) Request No. 2014-SA-0147 ANSES (2015) Request No. 2015-SA-0015

* Biomolecular Engineering Commission (CGB), replaced under the Act of 25 June 2008 by the High Council for Biotechnology (HCB).
** Assessments of risks to human and animal health were carried out by the French Food Safety Agency (AFSSA) until 2010, and have been performed by ANSES since then. On 1 January 2022, pursuant to Order No. 2021-1325 of 13 October 2021 and Decree No. 2021-1905 of 30 December 2021, ANSES took over the missions of the HCB concerning assessment of risks to the environment and public health of all uses of biotechnologies in the open environment.

The environmental risk assessment carried out in connection with an application for marketing authorisation under Regulation (EC) No 1829/2003 consists of an assessment of the potential risks, direct or indirect, immediate or delayed, to human health and the environment from the placing on the market of the GMO in question. It takes into account the characteristics of the GMO (recipient organism, genetic modification), the extent of its intended use, and also the conditions under which environmental exposure to the GMO is possible: (1) exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals given GM feed and of bacteria found in environments exposed to the faeces; (2) "accidental" release into the environment of viable seeds from these GMOs (i.e. during transport and/or processing).

Consequently, for each marketing authorisation for a GMO and as required by Regulation (EC) No 1829/2003, referring to the provisions of Directive 2001/18/EC, there must be a post-authorisation monitoring plan for the environmental effects of the GMO[9]. This monitoring plan shall meet the requirements of Annex VII of Directive 2001/18/EC, with the aim, mainly for authorisation holders and operators handling GMOs, of monitoring the occurrence of adverse health or environmental effects of the GMO that were not anticipated in the risk assessment. Accordingly, monitoring reports must be submitted each year by the GMO marketing authorisation holders.

In addition, Article 4.5 of Directive 2001/18/EC states that "*In the event of a release of GMO(s) or placing on the market as or in products for which no authorisation was given, the Member State concerned shall ensure that necessary measures are taken to terminate the release or placing on the market*".

ANSES's opinion was sought in this regulatory context, which prescribes post-authorisation environmental monitoring of GM oilseed rape authorised under Regulation (EC) No 1829/2003, in order to support the competent authorities in putting in place suitable management measures to stop "accidental" release.

[9] Available online on the website of the Community Register of GM Food and Feed: https://webgate.ec.europa.eu/dyna2/gm-register/

3.1.2. Monitoring plans related to marketing authorisation decisions for GM oilseed rape

As part of the marketing authorisations for GM oilseed rape in the EU granted under Regulation (EC) No 1829/2003 for import, processing and use as food or feed, or for any other use except cultivation, a monitoring plan for environmental effects must be implemented in order to identify the occurrence of any adverse effects. It should be noted that all marketing authorisations for GM oilseed rape include a similar monitoring plan.

For example, the monitoring plan associated with Implementing Decision (EU) 2015/687 authorising the placing on the market of products containing, consisting of, or produced from genetically modified oilseed rape MON 88302 states that environmental exposure due to unintentional release of GM oilseed rape seeds, "*which could occur for example via substantial losses during loading/unloading of the viable commodity* [10] (...) *can be controlled by clean up measures and the application of current practices used for the control of any adventitious oilseed rape plants, such as manual or mechanical removal and the application of herbicides with the exception of glyphosate*[11]*"*.

It requires a "*general surveillance*", "*proportionate to the extent of imports of the oilseed rape*", "*for the duration of the authorisation*". This monitoring is the responsibility of the marketing authorisation holder for the GM oilseed rape, represented by EuropaBio (European Association of Bioindustries), which must in particular "*ensure that the monitoring plan is put in place and properly implemented in accordance with the conditions of the authorisation*". This monitoring plan must also apply to all operators handling viable GM oilseed rape, represented by the trade organisations Coceral (European importers), Unistock (European silo operators) and Fediol (European processors). These organisations are on the front line and need to be vigilant in reporting any discrepancies observed as part of routine monitoring of the products handled and used.

The plan requires these organisations to remind their member companies, on an annual basis, that "*in the framework of their management or safety standards (ISO, HACCP, ….), procedures must be put in place and implemented to limit loss and spillage of viable oilseed rape and to routinely eradicate adventitious populations on their premises – any such adventitious population resisting routine eradication procedures, shall be treated as a potential adverse effect*". Companies must also "*report back any adverse effects reported to them to the European trade organisations, including during seed inland transportation*".

The monitoring reports on environmental effects, related to the marketing authorisations for these GM varieties of oilseed rape (until 2021), have not identified any adverse effects corresponding to the presence of plants resistant to routine eradication procedures.

[10] Pollen or seeds with the ability to germinate
[11] Here, each monitoring plan specifies which herbicides are to be excluded, according to the resistance conferred by the genetic modification of the GM oilseed rape to which it refers.

3.1.3. Implementation of the monitoring plan in the context of the formal request

According to the DGAL, given the absence of any GM oilseed rape cultivation in France, the presence by the roadside of oilseed rape identified as genetically modified could be due to releases of imported seeds during their transport to a nearby crushing facility.

Searches carried out by the competent authorities identified the Saipol crushing facility in the industrial port area of Grand-Couronne (port suburb of Rouen, Seine-Maritime, Normandy) as being involved in the GM oilseed rape plants that were found. According to data provided by the DGAL, this facility has been importing GM oilseed rape seeds since 2016.

Due to its GM oilseed rape seed import and crushing activities, the Saipol Group is subject to obligations regarding the implementation of procedures to limit loss and spillage of viable oilseed rape, and the systematic eradication of adventitious populations on its sites. Outside its sites, beyond the vicinity of its premises, Saipol is not responsible for the eradication of GM oilseed rape populations, but is required to put in place procedures to prevent and limit the release of seeds during transport. According to the information provided by the DGAL, the Haropa Port authority is responsible for eradicating GM oilseed rape plants located outside the Saipol site, in the port area.

The Saipol manufacturing facility in Grand-Couronne relies on numerous operators to import GM oilseed rape seeds to its site:

- Port terminal operators;
- The silo operator;
- The freight company, for barrowing the oilseed rape seeds;
- The company of freight drivers;
- The company in charge of monitoring handling operations (unloading the import ships), barrowing seeds (monitoring the trucks used for this purpose on the road) and taking samples from ships;
- The company in charge of maintaining green spaces;
- The service provider used for cleaning and weeding.

All of these service providers work on behalf of Saipol during its import operations. According to the information provided by the DGAL, since 2016 Saipol has put in place a set of internal procedures and instructions for its operators to manage the risk associated with the accidental release of GM oilseed rape seeds into the environment.

It should be noted that Saipol and some of its service providers are affiliated to one of the professional organisations involved in implementing the aforementioned monitoring plan for authorised GM oilseed rape.

3.2. Identification of GM oilseed rape in the environment

3.2.1. Background on the presence of GM oilseed rape seeds

In 2020, the top ten oilseed rape-producing countries were Canada, China, India, Germany, France, Poland, Russia, Ukraine, Australia and the USA, which account for about 85% of the world's production, estimated at over 72,375,000 tonnes (according to FAOStat[12]). In 2019, 27% of oilseed rape grown was genetically modified (according to the ISAAA report[13]). Imports of GM oilseed rape into Europe mainly come from Canada, Australia and to a lesser extent the USA (Sohn *et al*., 2021). Between July 2020 and June 2021, France imported 1,562,676 tonnes of oilseed rape (GM and non-GM), making it the largest importer of oilseed rape in the EU, ahead of Belgium, Germany and the Netherlands (Eurostat 2021).

Oilseed rape is one of the main crops used for producing oil in Europe. The rapeseeds are mainly processed into oil and meal, a by-product of oil extraction. Rapeseed oil is used for food, feed and biofuel production, while rapeseed meal is mainly used for animal feed.

The Saipol manufacturing facility in Grand-Couronne specialises in the processing of oilseed rape seeds and other oilseeds (erucic acid rapeseed grown in the region) for the production of edible vegetable oils, proteins for animal feed (rapeseed meal), biofuels (biodiesel, etc.) and vegetable glycerine[14].

The Saipol facility has been importing GM oilseed rape seeds since 2016 from Canada or Australia (exceptionally in 2022), as well as non-GM oilseed rape from the UK, Ukraine, Latvia, Lithuania and Romania. It also sources non-GM oilseed rape seeds grown in France.

The volumes of GM oilseed rape seeds imported by the Saipol facility have increased since 2016 (information protected by commercial secrecy – Figure 1). These GM oilseed rapeseeds are used specifically for the production of biofuels and the resulting meal.

[12] http://www.fao.org/faostat/en/#home
[13] International Service for the Acquisition of Agri-biotech Applications (https://www.isaaa.org/resources)
[14] According to the information on the Saipol Group website, available online: https://www.saipol.com/

Figure 1: Annual volumes of oilseed rape seeds imported by the Saipol Grand-Couronne manufacturing facility (*Source: DGAL, information communicated by the DRAAF/SRAL Normandy collected as part of a documentary investigation of the facility*)

According to the data provided by the DGAL, the transformation events[15] most commonly found in GM oilseed rape seeds imported by the Saipol facility between 2018 and 2022 were as follows:

- Ms8 in 98% of shipments;
- GT73 in 98% of shipments;
- Rf3 in 93% of shipments;
- MON 88302 in 76% of shipments;
- T45 in 5% of shipments.

Imported oilseed rape seeds are transported by ship and unloaded at two port terminals: primarily at Port Terminal 1 and, when this becomes saturated, at Port Terminal 2. The first terminal is located in the immediate vicinity of the Saipol facility in Grand-Couronne, while the second terminal is located in Rouen, about 10 km to the north (Figure 2).

From Port Terminal 1, the seeds are transported over a few dozen metres to the storage bins (silo operators) by a conveyor system or, exceptionally, by barrowing (using dump trucks).

From Port Terminal 2, the seeds are transported over around 10 kilometres to the storage bins exclusively by barrowing.

As the storage silo site is adjacent to the Saipol facility, transport between the two sites is by a covered (closed) conveyor belt system.

[15] A transformation event is the insertion of a transgene at a specific site in the genome of a plant.

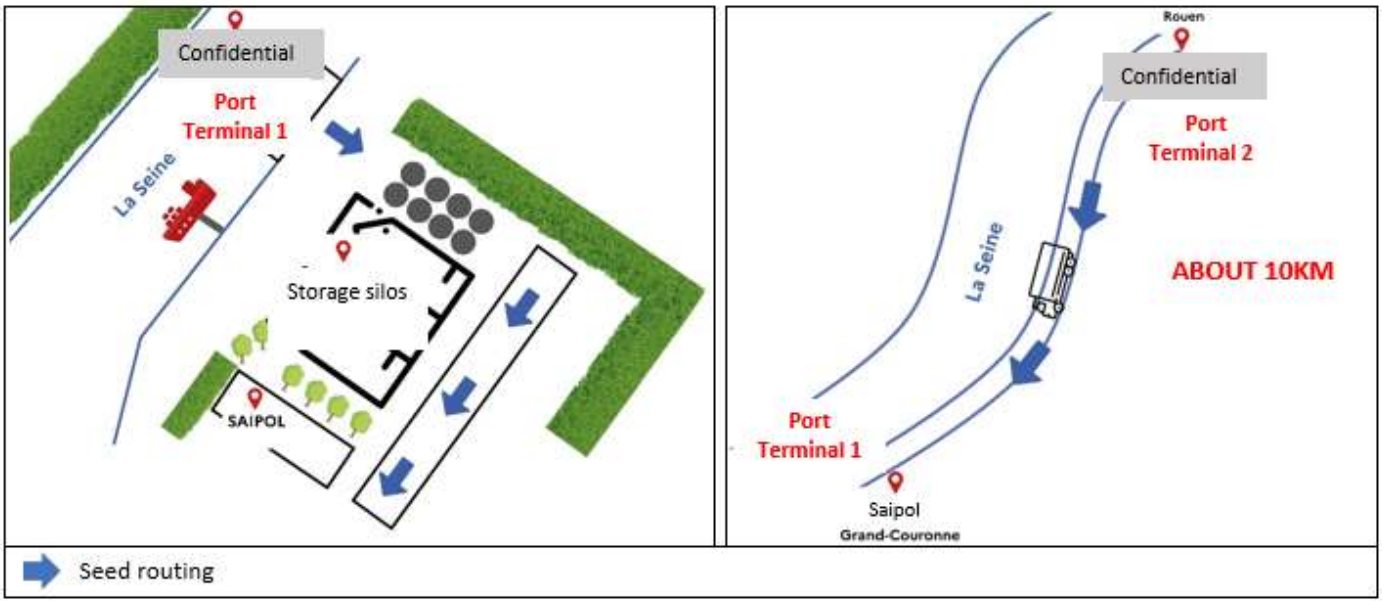

Figure 2: Geographical representation of the routing of oilseed rape seeds to the Saipol manufacturing facility in Grand-Couronne (ANSES 2023 diagram)

According to the data provided by the DGAL, between January 2021 and July 2022, four ships of GM oilseed rape seeds were unloaded at Port Terminal 2: two in March 2021, one in February 2022 and one in July 2022.

3.2.2. Geographical location and sampling of the identified oilseed rape plants

In late February 2022, a member of the Inf'OGM association happened to observe oilseed rape plants in flower by the side of the road in the port suburb of Rouen.

Samples were then taken of the observed oilseed rape plants, including stems, flowers, leaves and roots, and sent by the Inf'OGM association to the ADGène laboratory[16] for analysis. The results of these analyses, sent to the association on 6 April 2022, revealed the detection by real-time quantitative PCR of Ms8 and Rf3 transformation events. The nature of the samples was confirmed by the detection of the *ACC* reference gene for the oilseed rape species.

On 13 April 2022, the association informed the Ministry of Agriculture of this result, which was immediately acted on by the competent authorities, involving the Normandy Regional Directorate for Food, Agriculture & Forestry (DRAAF) and the Normandy Regional Food Service (SRAL). An inspection of the premises was carried out by the control services of these Directorates.

The inspection reports were sent by the DGAL and analysed by the ANSES WG on Biotechnology in the context of this formal request.

[16] A COFRAC ISO 17025/2017 accredited laboratory

Sampling was carried out by the inspection services on 19 and 29 April 2022 at the location mentioned by the association and for several kilometres along the roadside, as well as in and around the Saipol facility in Grand-Couronne (Figure 3).

The sample collection method was as follows: One large leaf or two small non-senescent leaves were collected from a maximum of 50 plants per sample, with 1 sample corresponding to a single sampling site.

Sixteen samples were taken, two of which were from the Saipol site (Figure 3). In the latter case, the inspection service's report concluded that there was sparse oilseed rape regrowth at the flowering or near-flowering stage, mainly in inaccessible places (around equipment, kerbs, gravel areas).

The other samples were taken near the manufacturing facility and along the roadside between Port Terminal 2 and the Saipol facility. At least nine plants were in flower, and two in the rosette stage (Annex IV).

Figure 3: Location of samples taken by the control services (DRAAF/SRAL Normandy) in the south-western port suburb of Rouen (in red, the GM samples; in blue, the non-GM samples) (Images from DRAAF/SRAL Normandy)

Sample 27635 was taken from the outer right-hand edge of a roundabout exit, where there was a high density of oilseed rape plants.

The inspectors assumed that the seed spillage responsible for the regrowth observed by the roadside had occurred during transport, in a braking and turning area that could have caused a loss of goods.

Sample 27633 was collected in the immediate vicinity of the Saipol facility, under the conveyors used to route the seeds between Port Terminal 1 and the silo operator. The presence of regrowth, noted by the inspectors under these conveyors, indicates that the transport of goods by these conveyors could also be responsible for oilseed rape seed losses.

Moreover, in addition to the data provided, the inspectors observed a railway line running from the secondary terminal to the Saipol facility. Oilseed rape seedlings were found in the immediate vicinity of this railway (Annex 4). The website of silo operator X provides the following information for its Grand-Couronne site: "X provides logistics services for Saipol. The oilseed rape is received by truck, train or barge. The oilseed rape is stored and then delivered to the Saipol facility."

3.2.3. Analysis of the oilseed rape samples and detection of transformation events

The samples taken by the control services were sent to the BioGEVES laboratory on 20 April and 2 May 2022. The analyses were carried out from 20 to 26 April 2022 and from 3 to 12 May 2022.

First, the laboratory screened for the presence of GMOs using the usual target sequences: *p35s, Tnos, pat, bar* and *ctp2-cpsps*. It also sought the transformation event DP-073496-4. When the screening was positive, the transformation event(s) present in the sample were investigated using specific detection methods for GM oilseed rape events[17], validated by the European Union Reference Laboratory established under Regulation (EC) No 1829/2003. These methods are based on the detection, using real-time PCR, of a specific sequence of each transformation event, most often corresponding to the junction between the insert and its 5' or 3' flanking sequence.

Transformation events that are not currently authorised for import into the European Union but are authorised for cultivation in exporting countries (Australia, Canada), such as Ms11 oilseed rape, and transformation events whose authorisation has expired but that are still tolerated at trace levels of 0.1% in imports, such as Ms1, Rf1, Rf2 and Topas 19/2, were also screened for by the BioGEVES laboratory.

The BioGEVES laboratory detected the following transformation events: GT73, Ms8, Rf3 and MON 88302. All of these are contained in GM oilseed rape authorised for import, processing and food and feed use in the EU (Table 1). They correspond to the events found in the import ships of the Saipol manufacturing facility.

[17] Available online on the website of the Community Register of GM Food and Feed.

Considering that the analyses were carried out after extraction of leaf DNA from the entire sample received, i.e. from several mixed plants, it is not possible to confirm the exact nature of the identified GM oilseed rape, which could correspond to single or stacked transformation events.

Two of the 16 samples were negative following target gene screening (corresponding locations shown in blue in Figure 3):

- Sample 27615, which was taken north of Terminal 2, and not on the route between the terminal and the Saipol site.
- Sample 27640, taken on the route of the seed trucks.

All the other samples were positive for different events (corresponding locations shown in red in Figure 3).

The samples taken at the Saipol site that were positive for the transformation events Ms8, Rf3 and MON 88302 led the control services to report the Saipol manufacturing facility as "non-compliant" with implementation of the monitoring plan.

Indeed, the presence of GM oilseed rape plants on its site demonstrates that the Saipol facility has not effectively implemented the measures provided for in the environmental monitoring plans (described in Section 3.1.2.), designed to systematically eradicate adventitious populations found in the facilities of operators handling viable GM oilseed rape.

Since it began importing GM oilseed rape seeds, the Saipol facility has put in place the following internal procedures:

- Cleaning up spills and recycling viable GM waste;
- Complete weeding of the site twice a year (June and October).

According to the inspection report, the inspectors considered that these measures were appropriate but were unable to prevent the presence of regrowth at the flowering stage in the most inaccessible areas.

The oilseed rape plants found on the Saipol premises were removed by the company.

The samples taken outside the facility's premises that were positive for the Ms8, Rf3, GT73 and MON 88302 transformation events indicated that the procedures put in place by Saipol to limit the release of seeds during transport were insufficient or incorrectly implemented. These procedures include the following:

- The bulk-handling crane grabs used for unloading the seed import ships must be self-sealing to avoid leakage, and the unloading docks must be cleaned regularly, and specifically after unloading each ship containing GM oilseed rape seeds, in order to remove any seeds that have fallen to the ground. The seeds must then be disposed of using specific destruction processes to ensure that no germination is possible;
- All GMO handling equipment must be cleaned after each use;
- Dump trucks used for barrowing must not allow any leakage and must be cleaned beforehand (residues swept up and then washed down with water). They must be covered with tarpaulins during journeys (even when empty) and filled to no more than 80%. A limited number of drivers is used, and they are trained to follow procedures.

- The monitoring company must ensure that measures are taken to limit the release of seeds from unloading and barrowing.

Saipol uses a large number of service providers for the unloading, transport, storage and monitoring activities subject to these different procedures (Section 3.1.3.).

The ANSES WG on Biotechnology believes that the data on the location of the oilseed rape plants sampled and their analysis as part of the site inspection by the control services have made it possible to identify a release of GM oilseed rape seeds directly linked to the activities of an operator that imports and processes these seeds.

The presence of GM oilseed rape plants on this operator's premises, as identified by the inspection service of the competent authorities, shows that the operator's internal procedures do not allow it to comply with the monitoring plan provided for as part of the authorisation of these GM oilseed rape plants and avoid the release of seeds and the presence of GM oilseed rape plants.

The monitoring plan also requires the operator to put in place procedures for external service providers, to limit the release of seeds. The presence of GM oilseed rape plants on the roads of the Rouen – Grand-Couronne industrial port area, leading to the operator's facilities, was observed by the Inf'OGM association and confirmed by the inspection service.

The ANSES WG on Biotechnology believes that the large number of service providers involved in the various stages of transporting the seeds to the operator's manufacturing facility (unloading, transport, storage, cleaning, weeding) makes it more difficult to control these procedures.

3.3. Assessment of the environmental risks associated with the "accidental" release of GM oilseed rape

3.3.1. Specific hazard analysis of the biological characteristics of oilseed rape

- Description of oilseed rape

Oilseed rape (*Brassica napus L.*) is an annual brassica with yellow flowers that is a natural cross between a cabbage (*B. oleracea*) and a turnip (*B. rapa*). Molecular dating indicates that this species appeared ~7500 years ago at most (Chalhoub *et al.* 2014).

The Brassicaceae are dicotyledonous plants, mostly herbaceous, whose four cross-shaped flower petals have led to the description "cruciferous". This family includes 430 genera and 3600 species. Many *Brassica* species have been domesticated, as the genus is highly polymorphic and has benefits such as the production of edible vegetable oil and vegetables. Their high levels of glucosinolates (secondary metabolites acting as a means of defence against pests) specific to *Brassica* and of erucic acid (a fatty acid that is harmful to health) previously limited their use for human consumption. Crosses between varieties have made it possible to obtain so-called "double-zero" cultivars: without erucic acid or glucosinolates.

Two types of oilseed rape[18] are grown, depending on the country:

- winter oilseed rape, with a long cycle (330-360 days) (Figure 4), mostly grown in Europe, including France. It needs low temperatures and short winter days for vernalisation in order to be able to flower in spring. The seeds are sown between late August and early September; they then germinate during the autumn and develop taproots and short leaves to reach the rosette stage with which they overwinter (vernalisation period). Stem elongation occurs in the spring. Flowering begins before the stem is fully elongated, around April, and lasts for over a month. The siliques (seed pods) are formed while flowering is still in progress, in early summer. At maturity, the siliques are more or less dehiscent[19] depending on the variety, and can burst and each release around twenty seeds a few metres away from the mother plant.

- spring oilseed rape, also called canola, with a short cycle (3-7 months) (Figure 4) is widely grown in Canada, the USA and Australia, where the harsh winter conditions do not allow the plant to overwinter. Unlike winter rape, therefore, spring oilseed rape does not need vernalisation to be able to flower and produce seeds. The seeds are sown as soon as the soil is warm enough, around March. The plant flowers in June and the siliques mature around August.

[18] There is also a semi-winter type of oilseed rape, selected and grown mainly in China.
[19] Dehiscence is spontaneous opening at maturity in order to release the seeds

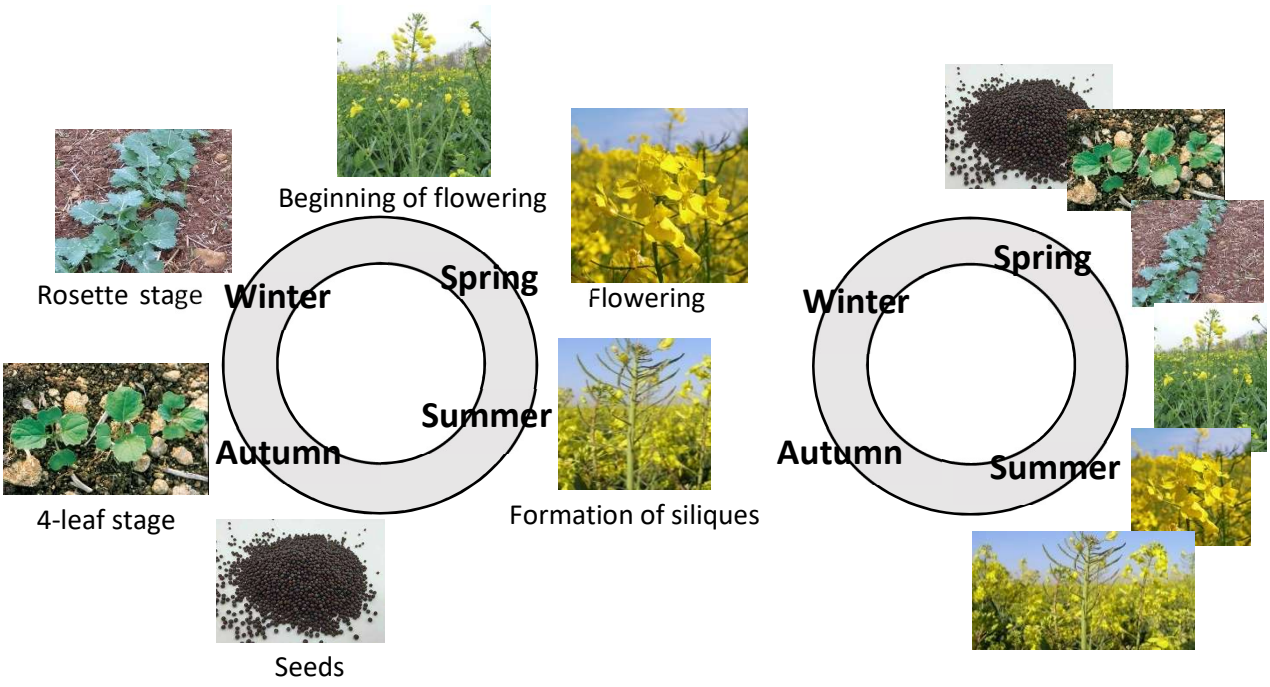

Figure 4: Cycle of winter (left) and spring (right) oilseed rape in cultivation (ANSES 2023 photomontage)

- Description and hybridisation risk of species related to oilseed rape

The species taxonomically related to *B. napus* (genome AACC, 2n=38) with the highest sexual compatibility – *B. rapa* (genome AA, 2n=20, self-incompatible), *B. oleracea* (genome CC, 2n=18, self-incompatible) and *B. juncea* (genome AABB, 2n=36) (self-compatible) – are also found in Europe. *B. juncea* varieties are mainly grown in France for their use in mustard production. The self-incompatibility of *B. rapa* and *B. oleracea* means they are unable to produce seeds from their own pollen. This factor favours interspecific crosses. The spontaneous formation of viable hybrids between these species and *B. napus*, at variable frequencies depending on the distance between the plants as well as the relative proportion of the species in the environment, is described in the literature (Liu *et al.* 2013; Halfhill *et al.*, 2004).

Brassica oleracea is a species of the Brassicaceae family, generally biennial, with several subspecies cultivated for their agronomic, economic and food value (cabbage, broccoli, cauliflower, Brussels sprouts, etc.). The plant grows from a shallow, fibrous taproot. The first petiolate leaves are arranged in a rosette of 7 to 15 leaves. Subsequent leaves have shorter petioles and develop by turning inwards to form a compact ball or "head". In cultivation, *Brassica oleracea* is harvested when the head is well formed and sufficiently dense, and before the flowers appear (except for seed production). Flowering occurs in the second year, in spring (May-June), then siliques are formed that open at maturity by dehiscence. *Brassica oleracea* is a cross-pollinated entomophilous (insect-pollinated) species.

The formation of spontaneous hybrids between *B. napus* and *B. oleracea* was detected in wild populations of *B. oleracea* using specific microsatellite markers (Ford *et al*., 2006). This was at two sites where *B. napus* was grown in close proximity (no more than about 25 m) to natural populations of *B. oleracea* on cliff tops in the UK. The frequency of hybrids resulting from pollination of *B. juncea* by oilseed rape seems to be 1.67%, while the frequency of reverse hybrids is 0.56% (Ford *et al*., 2015).

Growing hybrids under experimental conditions has shown that these would however often be sterile (low seed production), probably due to the difference in genome size between *B. napus* and *B. oleracea*. Nevertheless, subsequent generations may have a greater ability to produce seeds (Kaminski *et al*., 2020).

Brassica rapa is an annual or biennial, sub-cosmopolitan species. It has several subspecies, some of which are of agronomic, food or medicinal interest. There are three groups of *B. rapa*, defined according to their morphological characteristics: The oleifera or oilseed group (forage turnip); the leafy type including *Chinensis* (pak choy, celery mustard), *Pekinensis* (petsai) and *perviridis* (tender greens); and the rapifera type, which includes *rapifera* (turnip, rapini), as well as *ruvo* (broccoli rabe Italian turnip) (Bailey and Bailey, 1976; Prakash and Hinata, 1980)

B. rapa is cross-pollinated. The crop is harvested before flowering (except for seed production). The flowering period varies between groups of *B. rapa*.

Hybridisation between *B. rapa* and *B. napus* has been described in the literature. Its frequency varied between 1% and 17% (Simard *et al*., 2006; Warwick *et al*., 2003). All hybrids were morphologically similar to *B. rapa* and were triploid, but had 55% lower pollen viability than *B. rapa* and were thus less fertile than the parent plants. However, they were able to backcross with *B. rapa* and become diploid again, or even self-pollinate. Their fitness was generally intermediate to that of the parents. (Warwick *et al*., 2003; Bailleul, 2012). Genetic recombination between the genomes of *B. napus* and *B. rapa* easily permits introgression of oilseed rape genes (Leflon *et al*., 2007; Leflon *et al*., 2010). The frequencies of crosses of *B. napus* with *B. rapa* depend on the genotypes of the parents, agricultural practices and the density of the parent populations.

In Canada, gene flows between glyphosate-resistant GM oilseed rape and wild populations of *B. rapa* have been documented in several studies (Warwick *et al*., 2008; Yoshimura *et al*., 2016; Laforest *et al*., 2022). Persistence of the transgene in a *B. rapa* population was observed for six years in the absence of selection pressure from the herbicide (Warwick *et al*., 2008). Gene flow between cultivated oilseed rape and natural populations of *B. rapa* has also been reported in Argentina (Ureta *et al.* 2017). In the United Kingdom, on a national scale, hybrids were observed at a rate of 0.2% to 3% among populations of *B. rapa* occurring spontaneously along river banks or as weeds in crops, with the hybridisation rate decreasing as the distance between the *B. rapa* population and the cultivated oilseed rape increased (the distances reported in the study ranged from 30 to 3000 metres) (Wilkinson *et al.*, 2003). Most hybridisation seems to be in the direction of pollination of *B. rapa* by oilseed rape, but hybrids from pollination of oilseed rape by *B. rapa* have been observed in riverside populations, and more rarely in weed populations (Ford *et al*., 2015). In Japan, hybridisation has been observed

between transgenic oilseed rape resulting from seed dispersal during transport from the ports of entry and *B. rapa* plants from feral riverside populations[20] (Aono *et al.* 2011).

Brassica juncea is an annual amphidiploid species resulting from the natural crossing of *B. rapa* and *B. nigra*. *B. juncea* is widely grown in countries with subtropical and temperate climates including in Europe. *B. juncea* includes both vegetable and oilseed varieties. These seeds are commonly used in the food industry for their flavour, in flour or as a condiment, including table mustard. Almost 90% of French *Brassica juncea* (brown mustard) seeds are produced in Burgundy. Flowering occurs from May to July after a period of vernalisation at low temperatures.

B. juncea is mainly self-pollinating, but outcrossing rates of 20-30% have been reported (Rakow and Woods, 1987). Bees are then the main pollinators, as the heavy, sticky pollen cannot be carried over long distances by the wind.

In Japan, an analysis of the progeny of *B. juncea* plants located in areas where transgenic oilseed rape plants from accidental release were observed did not find any hybrids (Aono *et al*., 2006; Aono et *al*., 2011). The cases of "spontaneous" hybridisation between *B. napus* and *B. juncea* reported in the literature came from experiments in which the two species were grown in close proximity to each other (Séguin-Swartz *et al.*, 2013 in Canada; Tsuda *et al*., 2012 in Japan). Hybridisation rates ranged from 0% to 1.6%, depending on the conditions (distance between plants and flowering synchrony). These hybrids had high fitness and produced about 34% of viable pollen, allowing self-pollination and the production of an F2 generation or even outcrossing: F1 hybrids receiving *B. napus* pollen produced twice as many seeds as those receiving *B. juncea* pollen (Liu *et al.*, 2010).

For other species taxonomically related to oilseed rape (*Brassica nigra* and *Brassica carinata*), there is a very low possibility of producing viable hybrids. The fitness of F1 hybrids is considerably lower than that of the parents, with delayed and very low emergence rates (17% of the parents) and twice the mortality of the parent species (Guéritaine *et al*., 2002).

For *Brassica nigra*, studies indicate a very low probability or lack of success in producing viable hybrids between *B. napus* and *B. nigra*, due to the lack of a common chromosome set as well as differences in ploidy level: interspecific hybridisation and subsequent introgression between them under field conditions would therefore be possible, although very difficult (Pradhan *et al.,* 2010; Londo *et al.*, 2011; Bing *et al.*, 1996; Devos *et al*., 2009; Jahier *et al*., 1989).

Viable hybrids between *B. carinata* and *B. napus* were obtained by experimental crosses (FitzJohn *et al*., 2007). The frequency of hybridisation was low and the F1 hybrids were sterile (Getinet *et al.,* 1997).

[20] Wild population, growing outside cultivated fields and capable of self-perpetuation.

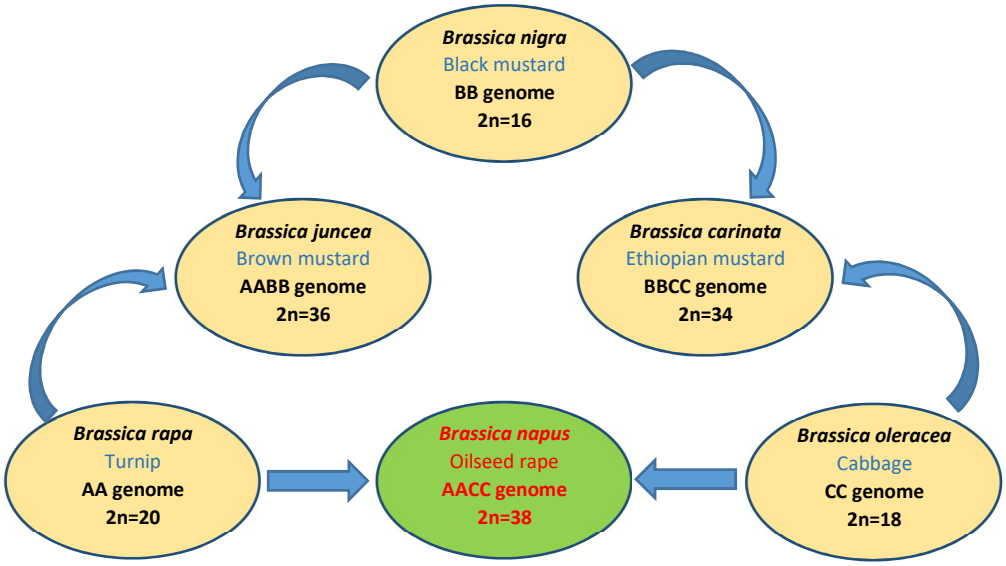

Figure 5: Genetic relationship between different Brassica species ("Triangle of U", described by Nagaharu, U., 1935)

Other cultivated and wild species of Brassicaceae can naturally hybridise with oilseed rape, such as *Raphanus raphanistrum* (wild radish), *Sinapis arvensis* (wild mustard), *Erucastrum gallicum* (hairy rocket) and *Hirschfeldia incana* (hoary mustard).

Raphanus raphanistrum and *Sinapis arvensis* are the two main weeds of oilseed rape (Chèvre *et al*., 2004).

Hirschfeldia incana can be found in fields of oilseed rape, with which it can hybridise, but the oilseed rape genome seems to be eliminated in its progeny (Chèvre *et al*., 1996; Darmency and Fleury, 2000).

For *Raphanus raphanistrum*, hybridisation is quite rare although it has been demonstrated experimentally in Canada (Warwick *et al*., 2003), Australia (Rieger *et al*., 2001) and France (Chèvre *et al.*, 2000) at similar frequencies of 10^{-5} to 10^{-7}. While first-generation hybrids have limited fertility (pollen viability of 0.12%), the plants regain fertility over several generations of pollination by *Raphanus raphanistrum* (Chèvre *et al*., 1997) and reach the fertility of the wild parents after three crosses. Furthermore, recent work (Adamczyk-Chauvat *et al*., 2017) indicates that the genes carried by oilseed rape can be stably integrated into the *Raphanus raphanistrum* genome, but that these exchanges between genomes are highly dependent on the initial position of the genes on the oilseed rape chromosomes.

Very recently, cases of spontaneous hybridisation between *B. napus* and *R. raphanistrum* in the Canadian environment were reported (Laforest *et al.,* 2022).

For *Sinapis arvensis*, hybridisation with oilseed rape is rare and it has not been possible to study the progeny of hybrids (Chèvre *et al*., 1996; Warwick *et al*., 2003)

The table below presents the current state of knowledge on the probability of genetic hybridisation between *B. napus* and its wild relatives, according to Liu *et al*.

Table 2: Probability of genetic hybridisation between *B. napus* and its wild relatives

Related species to *B. napus*	Probability of genetic hybridisation with *B. napus*
Brassica rapa	Likely
Brassica juncea	Likely
Brassica oleracea	Likely
Brassica nigra	Very unlikely
Raphanus raphanistrum	Very unlikely
Hirschfeldia incana	Very unlikely
Other related species	Unlikely

- Seed characteristics and germination conditions

Oilseed rape plants bear fruits called siliques, containing a single row of 15 to 25 small (1.8 to 2.7 mm) spherical seeds; the weight of 1000 seeds varies from 2.7 to 3.6 g (Gulden *et al.*, 2008). Each oilseed rape plant can produce as many as over 10,000 seeds (Garratt *et al*., 2018, study of winter oilseed rape). Little is known about seed production in feral plants (Gulden *et al.*, 2008). The number of siliques per plant depends on plant density (Gulden *et al*., 2017). Data from agronomic trials in North America and Canada show a variation ranging from 600 siliques for a single plant to fewer than 100 siliques per plant when there are above 50 plants per m^2 (Assefa *et al*., 2018).

There are two types of seed dormancy in plants. Primary dormancy occurs during seed maturation and during a period known as "post-maturation". It makes it impossible for the seeds to germinate immediately after they have been released. In oilseed rape, there is no or very little primary dormancy (Soltani *et al.,* 2019). Secondary dormancy allows seeds buried in the soil to remain viable for several years, thus building up a seed bank in the soil. The entry into dormancy is determined by unfavourable germination conditions in the soil (oxygen or light deficiency, osmotic stress, etc.) (Schwabe *et al.*, 2019). Under agronomic conditions, it has been shown that the presence of oilseed rape regrowth and the constitution of a seed bank (through secondary dormancy) depend mainly on the cultivated variety and, to a lesser extent, on post-harvest tillage that may or may not bury the seeds in the soil (Gruber *et al.*, 2018).

Monitoring of agricultural fields showed a rapid decrease in the soil seed bank in the first three years after an oilseed rape crop (Gulden *et al*., 2008). However, a fraction of this seed bank can persist and cause oilseed rape regrowth in the medium to long term: up to four years in California (Munier *et al.*, 2012), seven years in Canada (Beckie and Warwick, 2010), 10 years in Sweden (D'Hertefeldt *et al*., 2008) and up to 17 years in Denmark (Jørgensen *et al.*, 2007). In Germany, regrowth of transgenic oilseed rape has been observed up to 15 years after cultivation at agronomic trial sites. The seed bank persists longer when seeds are buried deeper in the soil (10 cm or more; Gulden *et al*., 2017).

Oilseed rape seeds can only germinate if they are buried less than 10 cm deep. In France, the recommended seeding depth is 2 to 5 cm[21]. The lowest temperature at which germination can occur is 1 to 4°C depending on the variety, while the optimum temperature is 20 to 25°C (Gulden *et al*., 2008; Luo and Khan, 2018). Seeds that have completed germination can survive temperatures as low as -20°C (Gulden *et al*., 2008).

- Conditions necessary for growth and flowering of the plants

Frost can affect the survival of seedlings as well as the reproduction of oilseed rape. Frost tolerance at the seedling stage is a quantitative trait that varies between varieties and is mainly differentiated between spring varieties, which are sensitive, and winter varieties, which are more tolerant (Fiebelkorn and Rhaman, 2016; Wrucke *et al.*, 2019). Many factors are involved: temperature and duration of the frost period, humidity, seedling stage (sensitivity is higher at the cotyledon stage than at the 3-4 leaf stage), acclimatisation to the cold before the frost period (Fiebelkorn and Rhaman, 2016).

Winter rape overwinters at the rosette stage (Figure 4), when it has maximum tolerance to cold and can withstand temperatures of around -15°C. In spring, when flowering is initiated (from the stem elongation stage), damage is observed when temperatures fall below -5°C, with destruction of leaves and stems and abortion of flowers[22].

Spring oilseed rape is only sown after winter (Figure 4); any feral spring rape that germinates before or during winter will be destroyed by frost. If the winter is mild and it survives, it will continue its cycle without a winter break at the rosette stage, and its flowering date will depend mainly on the temperature but also on the photoperiod.

The flowering period varies widely between varieties: spring varieties do not need a vernalisation period to flower, whereas winter varieties require prolonged exposure to cold (Letijten *et al.,* 2018). The conditions necessary for vernalisation of winter varieties are a short day photoperiod and temperatures between 3 and 7°C for 5 to 10 weeks (Merrien, 2010).

[21] Information accessed on the Terres Inovia website. Available online: https://www.terresinovia.fr/
[22] Information accessed on the Terres Inovia website. Available online: https://www.terresinovia.fr/

- Seed dispersal

Oilseed rape has no specialised mechanisms for seed dispersal. The siliques open at maturity, especially in dry conditions, allowing dispersal over a limited distance of a few metres around the plant (Bailleul *et al*., 2020). In the field, the phenomenon of silique dehiscence at maturity leads to harvest losses typically of around 5% of the seeds produced (Gulden *et al*., 2003). Oilseed rape seeds can be dispersed by various "natural" vectors: anemochory (dispersal by wind) or hydrochory (dispersal by water).

Seed dispersal by wild animals (zoochory) is not documented in the literature. However, in Australia, where flocks of sheep are allowed to graze the stubble after harvest, viable oilseed rape seeds have been found in the faeces up to 5 days after ingestion (Stanton *et al*., 2003). Human activities also cause seed dispersal (Hodkinson and Thompson 1997; Lu 2008), either intentionally – from sowing, which brings new seeds into the ecosystem, or harvesting, which moves seeds out of the agro-ecosystem – or accidentally or by chance from seeds being spread within and outside fields by harvesters (Ghersa *et al.* 1993), seeds from mowed plants left on roadsides (Strykstra, Verweij and Bakker 1997), losses during crop transport in dump trucks (Crawley and Brown 1995; Yoshimura, Beckie and Matsuo 2006), seeds carried by trains or vehicles when held by mud stuck to the cars or tractor wheels (Lippe and Kowarik 2007a and 2007b), seeds displaced by air movements caused by moving vehicles (Garnier, Pivard and Lecomte 2008), or seeds in the tread soles of walkers' shoes (Clifford 1956; Wichmann *et al.,* 2009).

- Characteristics of feral populations

Oilseed rape regrowth is common in cultivated fields. Following seed dispersal events, oilseed rape plants can also potentially become established outside fields to form feral populations. Feral populations of oilseed rape have been observed in many parts of the world: Europe, North America, Japan, Australia and New Zealand (Devos *et al*., 2012; Sohn *et al*., 2021, 2022). The size of the populations observed varies widely, from a few plants to several thousand (Devos *et al*., 2012; Pascher *et al*., 2017). These populations establish themselves in ruderal habitats, i.e. semi-natural land that has been disturbed by humans: in particular, field edges, verges of roads and paths, ditches, railway lines, building sites, ports, seed handling and storage facilities, waste ground and fallow land. A summary of studies conducted at five sites in Europe (France, Germany, Denmark and the UK) showed that populations were maintained for at least three years (Squire *et al*., 2011). The longest persistence observed at a French site (Selommes, Loir-et-Cher) was eight years for a roadside population (Pessel *et al*., 2011).

Different processes can explain the persistence of populations over time: production of seeds intrinsic to the population, recurrent germination of seeds from a (dormant) local seed bank, or recurrent inputs of seeds by dispersal from cultivated fields, from other feral populations or from an external source (transport of seeds, industrial activity, etc.).

The fact that feral populations are commonly found along roadsides and in agricultural areas suggests that external inputs have a dominant role. For example, in a study in Canada (Knispel *et al*., 2010) the presence of roadside populations was strongly associated with traffic intensity and distance to the nearest grain silo. At the French site of Selommes (Loir-et-Cher, France), genetic analyses showed a link between the varieties grown in a given year and the genotypes of the plants in the feral populations observed the following year (Bailleul *et al.*, 2016), confirming the preponderance of recurrent inputs from agricultural fields.

However, external seed inputs have been ruled out in some cases: the population observed by Pessel *et al*. (2001) consisted of a cultivar that had not been grown for eight years. Monitoring of a population from a single dispersal event in Australia showed that seed production by established plants maintained the population for at least three years (Busi and Powles, 2016). A study of an agricultural area in Germany showed that 30% to 48% of feral populations produced seeds (Elling *et al*., 2009). The populations monitored in Austria by Pascher *et al*. (2017) all produced viable seeds.

Only one study has attempted to estimate the relative proportions of the different demographic processes, based on data from the Selommes agricultural area and using a statistical model (Pivard *et al*. 2008). The majority of the observed populations (35%-40%) were thought to be the result of seed being brought in from neighbouring fields at harvest time. About 15% of these populations may have been transported during harvesting and spilled along the roadsides. Recruitment from the local seed bank may also have accounted for 35%-40% of the populations, while recruitment from seeds produced *in situ* represented no more than 10% of the populations (Figure 6).

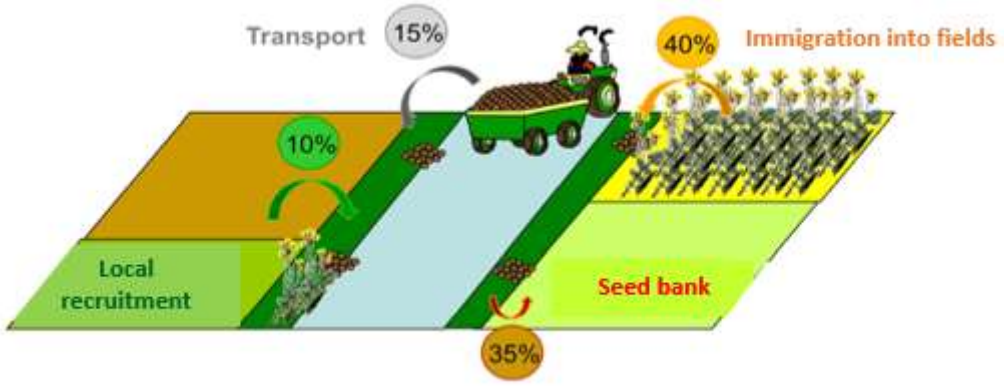

Figure 6: Different possible origins for feral populations in agrosystems (from Bailleul 2012; according to Pivard *et al.*, 2008)

- Gene flow from pollen dispersal

Oilseed rape has a mainly self-pollinating mode of reproduction. The outcrossing rate is about 30% and this cross-pollination is mainly from direct physical contact of the plants. However, pollen can also be carried by wind, insects (especially honeybees) and birds (Prakash *et al.,* 2011, Niemann *et al*., 2014, Gulden *et al.* 2008).

The effect of entomophilic pollination on seed production is not known with certainty, and it varies between oilseed rape varieties and between climatic and agronomic conditions (Ouvrard and Jacquemart, 2019). For example, Garratt *et al*. (2018) showed that the presence of insect pollinators only increases the amount of seeds produced when soil fertilisation is not limiting. When pollinating insects are abundant, the length of the flowering period is reduced and seed maturity is advanced. In the absence of insect pollination, the flowering period is extended, more flowers are produced and maturity is delayed.

Most of the pollen is dispersed within the first 50 metres and the frequency of gene flow decreases sharply thereafter. A wide variety of insects can disperse oilseed rape pollen. Fertilising pollen can be deposited by large pollinating insects at a distance of 1100 m from the original plant (Chifflet *et al*., 2011). Field studies report fertilising pollen dispersal distances (assessed using herbicide resistance as a genetic marker for gene flow) of up to 3 km (Cai *et al*., 2008; Devaux *et al*., 2008; Rieger *et al*., 2002). Indeed, wind gusts can carry viable pollen over several kilometres (Hüsken and Dietz Pfeilstetter, 2007). Devaux *et al.* (2007) showed long-distance gene flow of up to 5 km. In addition, Lankinen *et al*. (2018), showed that seeds are produced even in the presence of small amounts of pollen grains, suggesting that pollen flows between crops and feral plants may be possible under a wide range of conditions.

The gene flow from feral plants to cultivated fields is considered to be extremely low because in agricultural landscapes the number of feral plants is considerably lower than the number of cultivated plants (Devos *et al*., 2012). In a summary by Squire *et al*. (2011) concerning data from four agricultural areas in Europe, feral plants represented at most 0.002% of all flowering oilseed rape plants (two feral plants for every 100,000 cultivated plants).

3.3.2. Specific analysis of the risks associated with the biological characteristics of the GM oilseed rape containing the detected transformation events

- Oilseed rape containing the transformation event MON 88302

The oilseed rape MON 88302 was obtained by transforming hypocotyls of the Ebony cultivar with a disarmed strain of *Agrobacterium tumefaciens* containing the transformation plasmid. It expresses the 5-enolpyruvylshikimate-3-phosphate synthase enzyme of *Agrobacterium sp.* strain CP4 (CP4 EPSPS), which confers tolerance to the herbicidal active substance glyphosate. This oilseed rape is presented as a second-generation oilseed rape by Monsanto, which states that use of the *FMV/Tsf1* chimeric promoter enables expression of the CP4 EPSPS protein in pollen. This expression in pollen therefore improves weed control by enabling the application of plant protection products with higher concentrations of glyphosate and at later stages of development, without affecting male fertility.

In its Opinion of 3 July 2012[23], the HCB Scientific Committee had queried the adverse effects of the CP4 EPSPS protein on pollinator fauna, which were not documented. As there was no application for authorisation to cultivate this GM oilseed rape in the European Union, EFSA did not forward this request for additional information to the applicant.

The US Animal & Plant Health Inspection Service (APHIS) assessed oilseed rape MON 88302 as part of an application for a cultivation permit, and concluded that MON 88302 showed no toxic effects on animals or pollinators of other plants in or around fields cultivated with MON 88302, and that it was unlikely insects and animals would be significantly affected (Aphis, 2013).

Considering that:

- CP4 EPSPS is a 5-enolpyruvyl-3-phosphoshikimic acid synthetase, an enzyme that shares 46% homology with the EPSPS enzyme in plants;
- CP4 EPSPS and EPSPS catalyse the aromatic amino acid biosynthesis reaction (shikimate pathway). EPSPS is an enzyme naturally occurring in plants and bacteria. When its activity is inhibited by glyphosate, this causes aromatic amino acid synthesis to stop and leads to the death of the plant or bacterium;
- the CP4 mutated form comes from *Agrobacterium sp.* strain CP4, which is ubiquitous in the environment and not known to be pathogenic to animals or humans (Annex III of Directive 2000/54/EC[24] and the HCB Scientific Committee opinion of 9 September 2011[25]). CP4 EPSPS is naturally insensitive to glyphosate. However, glyphosate can bind to CP4 EPSPS in a condensed, non-inhibitory conformation (Funke *et al*., 2006);
- the EPSPS enzyme is present in the bacteria of bee gut flora (Motta *et al.,* 2018);
- the CP4 EPSPS enzyme is unstable to heat and digestion: when subjected to *in vitro* proteolytic digestion tests, the enzyme was degraded in less than 15 seconds in a gastric digestion model and in less than 10 minutes in an intestinal digestion model (AFSSA opinion, 2003[26]);
- the level of expression of CP4 EPSPS in pollen is about 9 µg/g dry weight (compared to 27 µg/g in seeds or 230 µg/g in leaves) (Food Standards Australia New Zealand opinion on oilseed rape MON 88302, 2012);
- the eradication of GM oilseed rape plants provided for in the monitoring plan limits the exposure of pollinating insects to oilseed rape MON 88302.

The WG on Biotechnology believes that the risks associated with the exposure of pollinating insects to the CP4 EPSPS enzyme are negligible if the plants are eradicated before the flowering stage, in accordance with the measures provided for in the monitoring plan attached to the marketing authorisation.

[23] Opinion of the HCB Scientific Committee of 3 July 2012 on Dossier EFSA-GMO-BE-2011-101
[24] Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
[25] Opinion of the HCB Scientific Committee of 9 September 2011 on Dossier EFSA-GMO-NL-2005-24
[26] AFSSA opinion in response to Request No. 2003-SA-0027 on the placing on the market of seeds and products derived from glyphosate-tolerant (Roundup Ready) maize line NK603 under Regulation (EC) No 258/97)

The CP4 EPSPS protein is not toxic for human or animal consumption (EFSA Opinion, 2014). The transfer DNA (T-DNA) containing the *Cp4 epsps* gene expression cassette is present at an insertion locus and in a single copy. The trait is stable over generations of self-pollination and crosses. No transgenes other than those carried by the T-DNA are present in oilseed rape MON 88302. The insertion does not interrupt any known or recognisable coding or regulatory sequences of the oilseed rape. Studies by Monsanto (using Southern blot) have shown the inheritance of the insert over five generations of self-pollination, and segregation analyses (presence of the transgene detected by PCR) of populations from heterozygous crosses have shown that the transgene is passed on to the progeny as a dominant Mendelian trait.

The compositional values for oilseed rape MON 88302 are within the natural variability of non-GM reference varieties.

Significant differences were detected for three agronomic traits (seed maturity, lodging and days-to-first flowering). These differences are within the equivalence limits defined for conventional reference varieties, except for the delay in flowering: in the US, it takes an average of 63 days for 50% of oilseed rape MON 88302 plants to flower, compared with 58.7 days for the near-isogenic comparator, and equivalence limits for conventional reference varieties of 50.4 to 59.7 days. As this difference is only a few days, it is unlikely to change the selective value of the plants.

Regarding the environmental risks, EFSA (2014) concluded that in the event of accidental release into the environment of viable oilseed rape MON 88302 seeds, there are no indications of an increased likelihood of spread and establishment of oilseed rape MON 88302 plants or hybridising wild relatives unless these plants are exposed to glyphosate-based herbicides.

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions, in the absence of a glyphosate-based herbicide treatment.

Due to the bacterial origin of the *Cp4 epsps* gene, horizontal transfer of the gene to other bacteria is theoretically possible, by homologous or non-homologous recombination. However, this potential horizontal transfer to bacteria, including those in the gastrointestinal tract, would not pose a risk to the environment (EFSA, 2014).

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the negligible risk to the environment of horizontal gene transfer between oilseed rape MON 88302 and environmental bacteria, and believes it is unlikely that the *Cp4 epsps* gene will persist in the bacterial genome. Indeed, these new genes would not confer any advantage on the bacterium and would be lost due to the absence of any selection pressure. The risk of a "stable" transfer is therefore considered to be negligible.

- Oilseed rape containing the transformation event GT73

The genetically modified oilseed rape GT73 was obtained by transforming six-week-old leaves and buds of the Westar cultivar with a disarmed strain of *Agrobacterium tumefaciens* containing the transformation plasmid PV-BNGT03. The genetic modification introduced into the oilseed rape GT73 consists of two tandem genes enabling the expression of two proteins, CP4 EPSPS and GOXv247. These two proteins are expressed constitutively at low levels in both the leaves and seeds of the plant, and are not correlated with any particular stage of plant development. The two proteins confer tolerance to glyphosate by different molecular mechanisms: GOXv247 allows the degradation of glyphosate into glyoxylic acid and aminomethylphosphonic acid (AMPA), whereas CP4 EPSPS is insensitive to inhibition by glyphosate, unlike the endogenous EPSPS enzyme. No biologically relevant differences were identified in the composition or the agronomic and phenotypic characteristics of oilseed rape GT73 compared to its conventional counterpart, except for the newly expressed CP4 EPSPS and GOXv247 proteins. Neither protein is toxic for human or animal consumption, or potentially allergenic (EFSA Opinion, 2013).

Lastly, regarding the environmental risks, in its opinion of 2013, EFSA concluded that in the event of the accidental release into the environment of viable oilseed rape GT73 seeds, there are no indications of an increased likelihood of establishment and spread of oilseed rape GT73 plants or hybridising relatives, unless these plants were exposed to glyphosate-based herbicides.

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions, in the absence of a glyphosate-based herbicide treatment.

Owing to the authorised intended uses of oilseed rape GT73, the level of exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape GT73 is expected to be low. Due to the bacterial origin of the *Cp4 epsps* and *goxv247* genes, horizontal transfer of these genes to other bacteria is theoretically possible, by homologous or non-homologous recombination. However, this potential horizontal transfer to bacteria would not pose a risk to the environment (EFSA, 2013).

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the negligible risk to the environment of horizontal gene transfer between oilseed rape GT73 and environmental bacteria, and believes it is unlikely that these genes will persist in the bacterial genome. Indeed, these new genes would not confer any advantage on the bacterium and would be lost due to the absence of any selection pressure. The risk of a "stable" transfer is therefore considered to be negligible.

- Oilseed rape containing the transformation events Ms8 and Rf3

The oilseed rape Ms8 and Rf3 varieties were obtained by genetic modification of the Drakkar variety with *Agrobacterium tumefaciens*. The traits induced by the expression of these transgenes are stable over generations of self-pollination and crossing. No transgenes other than those carried by the T-DNAs are present in any of the oilseed rape.

The Ms8 event contains an expression cassette including the *barnase* gene conferring male sterility, and the *bar* gene conferring tolerance to glufosinate-ammonium herbicides. The *bar* gene, from the bacterium *Streptomyces hygroscopicus*, expresses the enzyme phosphinothricin acetyl transferase (PAT), which detoxifies glufosinate-ammonium. Oilseed rape Ms8 does not produce viable pollen and is therefore never self-pollinated.

The Rf3 event contains an expression cassette including the *barstar* male fertility restoration gene and the *bar* glufosinate-ammonium herbicide resistance gene.

Oilseed rape Ms8xRf3 has the herbicide resistance trait and male fertility trait, which is restored due to hybridisation between the two parent lines. Expression of the *barnase* and *barstar* genes has not been detected in seeds and pollen. In leaves and flower buds, *barnase* expression in Ms8 was below the limit of detection (0.1pg/µg total RNA). *Barstar* expression in Rf3 was only detected in flower buds (between 1.2 and 2.4 pg/µg total RNA). Expression of the *bar* gene in leaves and flower buds varied between 0.03 and 0.22 pg/µg RNA in Ms8, and between 0.2 and 1.1 pg/µg total RNA in Rf3. No expression of the *bar* gene was detected in pollen (CGB Opinion on Ms8, Rf3 and Ms8xRf3).

The composition of the oilseed rape Ms8, Rf3 and their hybrid Ms8xRf3 is within the variability of reference varieties. Regarding variety-specific secondary dormancy characteristics, regrowth of oilseed rape Ms8xRf3 for up to 13 years has been observed in Germany (Belter, 2016).

Lastly, regarding the environmental risks, in its opinion of 2012, EFSA concluded that in the event of the accidental release into the environment of viable oilseed rape Ms8, Rf3 and Ms8xRf3 seeds, there are no indications of an increased likelihood of establishment and spread of oilseed rape Ms8, Rf3 or Ms8xRf3 plants, or hybridising relatives, unless exposed to glufosinate-ammonium-containing herbicides.

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the risk of establishment of oilseed rape Ms8, Rf3 or Ms8xRf3 in the absence of a glufosinate-ammonium-based herbicide treatment.

Owing to the authorised intended uses of oilseed rape Ms8, Rf3 or Ms8xRf3, the level of exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape Ms8, Rf3 or Ms8xRf3 is expected to be low. Due to the bacterial origin of the *bar, barnase and barstar* genes, their horizontal transfer is theoretically possible, by homologous or non-homologous recombination. However, this potential horizontal transfer to bacteria would not pose a risk to the environment (EFSA, 2012).

The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the negligible risk to the environment of horizontal gene transfer between oilseed rape Ms8, Rf3 and Ms8xRf3 and environmental bacteria, and believes it is unlikely that these genes will persist in the bacterial genome. Indeed, these new genes would not confer any advantage on the bacterium and would be lost due to the absence of any selection pressure. The risk of a "stable" transfer is therefore considered to be negligible.

3.3.3. Characterisation of the context of exposure of the environment to GM oilseed rape plants

- Import volumes, period and frequency of release

The release of the GM oilseed rape seeds that caused the regrowth observed by the Inf'OGM association and the Ministry of Agriculture's inspection services in the industrial port environment near Rouen is very likely to have been caused mainly by losses due to the barrowing operations that transport the seed between Port Terminal 2 and the silo operator. As previously demonstrated, it could have also come from the conveyor transport between Port Terminal 1 and the silo operator (see Section 3.1.2.). These GM oilseed rape seed transport "events" have occurred several times a year: twice in March 2021, once in February 2022 and once in July 2022 (see Section 3.1.1.). The volumes of imported GM oilseed rape doubled between 2016 (219,616 tonnes) and 2020 (452,076 tonnes).

The seeds from the single and stacked event oilseed rape GM events (GT73, Ms8, Rf3 and MON 88302) identified in the environment had been imported by Saipol from countries where spring varieties of oilseed rape are grown. The identified GM oilseed rape plants could also have resulted from several seed dispersal events during transport.

Considering:

- **the variability of the spring oilseed rape cycle, which can extend from 3 to 6 months;**
- **the ability of seeds to germinate at several periods: mainly in spring and late summer, but potentially all year round;**
- **the possibility that the seeds may survive for several years due to the phenomenon of secondary dormancy;**
- **and that the GM oilseed rape plants observed in the spring of 2022 may not only be first-generation plants resulting from the germination of accidentally dispersed seeds, but also plants from later generations after reproduction;**

the ANSES WG on Biotechnology concludes that it is not possible to determine, from the available evidence, the date of the events that led to this release of GM oilseed rape seeds.

- Analysis of the environment where the GM oilseed rape was released

The environment where the GM oilseed rape plants were identified is an industrial port area on the banks of the Seine, between the city of Rouen, where Port Terminal 2 is located, and the town of Grand-Couronne, home to the Saipol manufacturing facility. There is plant cover on the side of the road connecting these two points, where the GM oilseed rape plants were identified. A railway track also runs along this section, separated from the road by a gravelled median with plant cover.

There is plant cover under the conveyors that transport the seeds between Port Terminal 1 and the silo site.

There are also areas of vegetation on the Saipol site.

At least one allotment garden was observed by the Ministry of Agriculture's inspection services near the oilseed rape plants, north of the commune of Petit-Couronne. The presence of allotments and the associated tillage of the soil could promote the burial of rape seeds and their entry into secondary dormancy.

The Inf'OGM association returned to the site in July 2022 and took new samples near the Saipol facility, without specifying any precise location. The association noted the presence of oilseed rape at the mature stage (with siliques). The sampled plants were sent to the Eurofins laboratory[27] on 26 July 2022. The analyses detected the Ms8 and Rf3 transformation events.

These mature plants were able to produce seeds, which could potentially be dispersed and buried, and then germinate either in the autumn of 2022 or spring of 2023, depending on weather conditions. In addition, the seeds may have entered secondary dormancy induced by various factors: road works, mowing, tillage on nearby allotments, burial by animals or cracks in the ground, leading to longer-term germination.

In this context, the ANSES WG on Biotechnology believes that, depending on soil disturbance and climatic conditions that are favourable or unfavourable to germination, the seeds could alternate cycles of dormancy and emergence from dormancy. New GM oilseed rape plants could therefore be observed for several years.

- Analysis of the environment near the GM oilseed rape release sites

Analysis of the environment in the vicinity (1 to 10 km) of the GM oilseed rape plants that were found identified winter oilseed rape crops within a 10 km radius of the Saipol Grand-Couronne site (Figure 7). The first agricultural fields planted with winter oilseed rape crops are located 5 km from the Saipol site. However, the nearest agricultural fields face the area, around 1 km away on the opposite bank of the Seine, and these grew oilseed rape in 2020.

These distances of 1 and 5 km mean that it is not possible to avoid the risk of gene flow through pollen dispersal, especially by large pollinating insects (Section 3.2.1.).

However, the ANSES WG on Biotechnology considers that as this release only concerned plants spread over very small areas, and not a GM oilseed rape crop, any

[27] A COFRAC ISO/CEI 17025 accredited laboratory

gene flow via pollen towards local, non-GM and very dominant crops could only lead to an extremely low contamination rate.

Furthermore, the ANSES WG on Biotechnology points out that according to the available data[28], there is no oilseed production in the Normandy region. The risk of seed contamination by GM oilseed rape due to the accidental release of these seeds is therefore considered to be negligible.

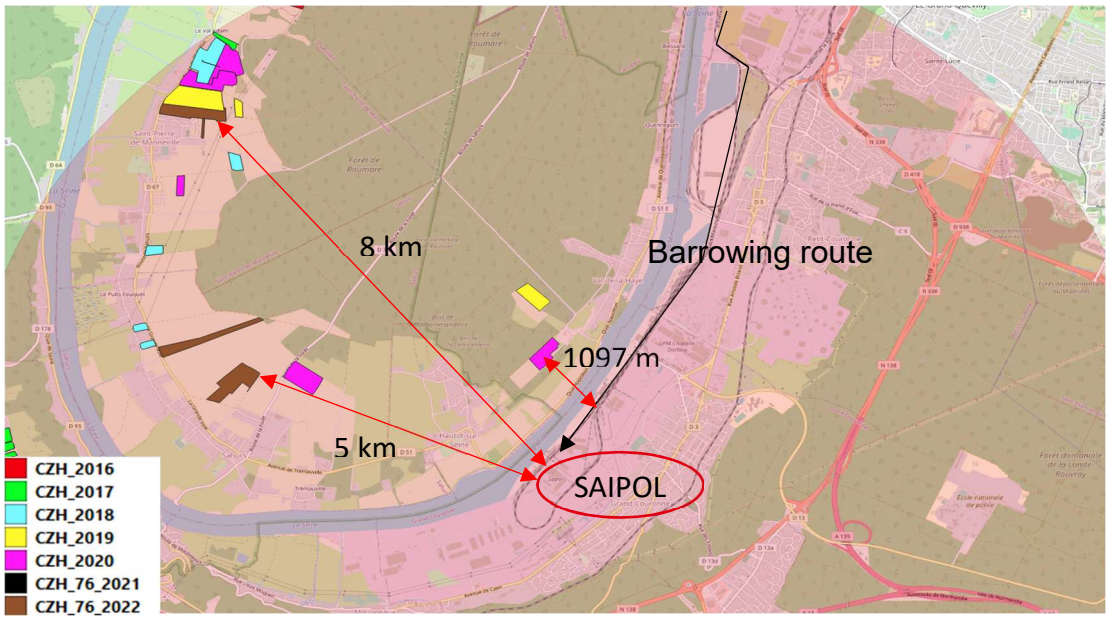

Figure 7: Agricultural fields of winter oilseed rape cultivation between 2016 and 2022 near the Saipol Grand-Couronne site (Images and distance calculation using QGIS software – data on the location of fields obtained from the DRAAF/SRAL Normandy)

Regarding the presence of species related to oilseed rape in the site environment, *Brassica rapa* (mainly turnip) and *Brassica oleracea* (mainly fodder cabbage) are grown in the region (Common Agricultural Policy (CAP) data – 2016 to 2022). However, these crops are harvested before the oilseed rape flowers appear, so there is no risk of gene flow.

Lastly, regarding the presence of spontaneous populations of these related species:

- According to a study (Maggioni *et al.*, 2020), wild forms of *B. oleracea* have been described in Normandy. However, they usually grow among cliffs on the coast. The likelihood of them coming into contact with cultivated fields is virtually zero (A.M. Chèvre, personal communication).
- For *B. rapa*, spontaneous (volunteer) forms are essentially regrowth of very old crops and are found in many regions in France, including Normandy. They usually grow on the edge of ditches (A.M. Chèvre, personal communication).

[28] According to data found on the SEMAE website on accepted areas under seed production – 2021 harvest: https://www.semae.fr/etudes-donnees-statistiques-semences/

- For *B. juncea*, there do not seem to be any spontaneous forms in France and the main area of cultivation is the Bourgogne-Franche-Comté region (A.M. Chèvre, personal communication).

Thus, among these species, considering the hybridisation rates of *B. napus* with its related species reported in the literature (see Section 3.2.1.) and the exposure context, the ANSES WG on Biotechnology considers that the highest hybridisation risk with GM *B. napus* plants would come from wild forms of *B. rapa*.

- Risk analysis of a selective advantage of GM oilseed rape plants

Only exposure of the plants to the herbicides to which they are resistant (glyphosate for the GT73 and MON 88302 transformation events; glufosinate-ammonium for the Ms8 and Rf3 transformation events) would confer a selective advantage to these GM oilseed rape plants. The use of glufosinate ammonium has been prohibited in France since 2017.

Glyphosate is contained in plant protection products authorised for various uses[29]:

- weeding of established crops,
- weeding of intercrops and set-aside land, and destruction of intermediate crops, in no-till situations only, except for spring crops planted after summer or early autumn ploughing in hydromorphic soils,
- weeding of trees and shrubs in open ground,
- weeding of grass lawns,
- weeding of railway tracks,
- weeding of industrial sites.

Article L253-7-II of the French Rural and Maritime Fishing Code states that it is forbidden for public entities (including public establishments) to use plant protection products (including glyphosate-based products) or cause them to be used for maintaining green spaces, forests, roads or walkways accessible or open to the public and belonging to their public or private domain.

Haropa Port is a public establishment. It is responsible for maintaining the entire road network in the port area. GM oilseed rape plants here will therefore not be exposed to glyphosate.

The railways in the industrial port area may be co-managed by the SNCF Réseau Group and the port authority Haropa Port. Article R5352-1 of the French Transport Code states that the connection agreement, signed between SNCF Réseau and the port authority in application of Article L.5351-5, defines the obligations and responsibilities of each of the parties with regard to their respective infrastructures. This agreement concerns maintenance or operating services performed by one party on behalf of the other. SNCF Réseau committed to stop using glyphosate from 2022[30].

[29] Non-exhaustive list, please refer to the ANSES catalogue of plant protection products and their uses: https://ephy.anses.fr/
[30] Information accessed from the SNCF Group website. Available online: https://www.sncf.com/fr/engagements/developpement-durable/maitriser-vegetation-respectant-environnement

In this context, the WG on Biotechnology considers that in the absence of any glyphosate or glufosinate-ammonium herbicide treatment, the released GM oilseed rape will not have any selective advantage at the sites examined in this request. However, the WG recommends that the competent authorities ensure that the commitments made by the SNCF Réseau Group to stop using glyphosate are properly implemented, particularly on the railways in the Rouen industrial port area. Indeed, glyphosate herbicide exposure of the identified GM oilseed rape plants located in the surrounding area, in particular on the areas of plant cover between the road and the railway line, could induce a selection pressure in their favour, thereby promoting their persistence in the environment.

Lastly, for the Saipol site, the recommendations of the "*Guideline for the management of Oilseed Rape Volunteers, including herbicide tolerant varieties*", drawn up by Monsanto SA and Bayer CropScience, should be applied, as described in the Saipol Group's procedures.

These guidelines reiterate that prevention of seed spillage and effective clean-up after spills are the basis of good seed handling management practices. For maintaining seed handling sites, the guidelines recommend avoiding unnecessary accumulation of litter, which provides a substrate for seed germination. They advise regular sweeping or brushing of surfaces to remove very small volunteer plants soon after they have germinated. When plants are at an advanced stage, the guidelines recommend manual or mechanical removal (mowing) or treatment with herbicides, avoiding the application of glyphosate (or glufosinate-ammonium, in countries where the substance is authorised) alone. Glyphosate can, however, be used in combination with other substances: the plants will then be destroyed and there can be no selection pressure.

The WG on Biotechnology warns about the fate of uprooted plants, collected seeds and other waste from GM plants. The WG believes that the management of such waste must ensure that there is no risk of release and germination of GM seeds.

3.3.4. Analysis of previous cases in the literature of "accidental" release of oilseed rape

Studies in many countries support the view that the main cause of release and establishment of feral oilseed rape plants is the loss of seed during transport by truck or train from production or import areas to processing plants (Sohn *et al.*, 2021). In France, dispersal during post-harvest transport has been documented by studies in the agricultural area of Selommes (Loir-et-Cher). Regarding the dispersal of transgenic oilseed rape seeds from import sites, two case studies seem particularly relevant: Switzerland and Japan.

- Dispersal by road transport during harvesting in France

In the study by Bailleul (2012), seed traps were placed along the roads serving the Selommes grain silo to measure losses during transport following the harvesting of oilseed rape fields. The seeds were transported by trucks in trailers without tarpaulins. The amount of seed lost increased with the size of the fields served and decreased with the distance from the fields. During the entire harvest period (8 days), an average of 400 seeds/m^2 were estimated to be scattered by the roadside. Genetic analyses attributed the dispersed seeds to their field of origin and enabled their dispersal distance to be estimated (Bailleul *et al*., 2020); this was 1250 m on average. Secondary dispersal of seeds on roadsides is also possible, mainly due to air turbulence caused by passing vehicles (Garnier *et al*., 2008). Thus, 20% of the seeds deposited by the roadside are scattered over a few metres. The dispersal distance increases with the road traffic, the maximum observed being 21.5 m (Garnier *et al*., 2008).

- Dispersal by road transport near ports of entry in Japan

Japan has never allowed the cultivation of GM oilseed rape. Conventional oilseed rape cultivation was widespread in Japan until the 1960s, when it almost disappeared. It currently covers less than 2000 ha (Sohn *et al*., 2021, 2022). However, feral populations of oilseed rape are still present, mainly along roadsides and rivers. Japan currently imports about 2.4 million tonnes of oilseed rape per year, mostly transgenic, and mainly from Canada (73%) and Australia (27%) (Sohn *et al*., 2021, 2022).

Transgenic oilseed rape plants resistant to glufosinate-ammonium or glyphosate were first reported in 2005 in the Kanto region (Saji *et al*., 2005; Aono *et al*., 2006). These plants were found in five major ports and along roadsides up to 40 km away. Doubly resistant plants have been observed, suggesting crosses between the imported transgenic varieties. Three years of monitoring (2006 to 2008) in the vicinity of 12 major ports in Japan subsequently revealed the presence of transgenic plants on roadsides near seven of the ports studied, and also on the riverside for one of them (Aono *et al*., 2011). A hybrid with *Brassica rapa* was detected on the river bank in this study. Systematic surveys were then conducted over three-year periods on two roads: one serving the port of Kashima (Route 51), and the other serving the port of Yokkaishi (Route 23) (Nishizawa *et al.*, 2009, Nishizawa *et al.*, 2016). In both studies, non-transgenic and transgenic feral plants were observed every year, although in varying numbers. These plants were detected on both sides of the road, suggesting either accidental losses on the trucks' journeys from the port as well as on their return journeys, or seed dispersal from one side of the road to the other. The plants were located in drains, ditches and cracks in the asphalt. Both flowering and mature plants were observed. Feral plants were observed almost all year round with a peak in spring (March to May). For a 19 km segment along Route 51, monitoring started in 2005 and was extended to 2014, i.e. 10 years of surveys (Nakajima *et al*., 2020). Depending on the year, the proportion of glyphosate-resistant plants ranged from 0% to 10.8% and glufosinate-resistant plants from 0% to 0.42%. Following road repairs, the total number of feral plants fell sharply from over 2000 in 2005 to 25 in 2014 (of which four were glyphosate resistant). Over the 10-year period, the areas with the highest densities of plants resulting from spillage were one with frequent hills, another where the road narrowed, probably inducing more vehicle acceleration and deceleration, and lastly a fenced plot of land where the plants could not be mown. The authors concluded from their study that there was

little likelihood of seeds dispersed along roadsides producing long-term expanding populations, as long as regular maintenance and repair work was carried out.

- Dispersal on railways and river ports in Switzerland

Switzerland has never authorised the cultivation of transgenic oilseed rape, and the import of transgenic seeds and plants has been prohibited since 2008. Switzerland does not import conventional oilseed rape from the main GM oilseed rape producing countries such as Canada or the USA, but it does import large quantities of wheat from Canada (Sohn *et al.*, 2021).

An initial study to detect glyphosate-resistant oilseed rape plants was conducted in 2011 and 2012 at a total of 79 railway sites (Schoenenberger and D'Andrea, 2012). Feral oilseed rape was observed at 58 sites (73%), and glyphosate-resistant oilseed rape was found at four sites. The frequency of these transgenic plants was only 2% at one of the sites but around 90% at the other three. A second study conducted in 2011 and 2012 (Hecht *et al.*, 2014) searched for the presence of plants containing the transformation event GT73, targeting two import areas for processing plants: the first area, Ticino, located on the border with Italy, included 36.7 km of railway; the second area, Basel, on the border with France, included 14.8 km of railway and two port sites on the Rhine. In Ticino, transgenic plants were found at the railway station in Lugano, where they accounted for 81% of the plants (22/27), and in the Basel area, both at the port of Basel (23% of the plants, 46/198) and at the Saint-Jean railway station (80%, 113/141). In 2013, a new analysis of the plants found in the Basel area revealed a second location at the port on the Rhine with GT73 transgenic plants, as well as five locations with glufosinate-resistant Ms8xRf3, Ms8 or Rf3 plants (Schulze *et al.* 2014). Crosses between GT73 plants and non-transgenic plants were also found. However, no crosses with related species were detected.

Transport of seeds from silos via the Basel Saint-Jean railway station came to an end in 2009. The feral population observed from 2011 to 2013 is therefore most likely to have originated in that period. In Switzerland, weeds on the railway tracks are controlled using a herbicide containing glyphosate, which may have given an advantage to the GT73 transgenic plants (Schulze *et al.*, 2014). In the Rhine port area, seeds were regularly being unloaded at the time of the studies (Schulze *et al.*, 2014). The aggregated distribution of transgenic plants at the port suggests that the release took place during the unloading of cargo.

Wheat is the main agricultural product imported into the Rhine port of Basel. Between 2010 and 2013, 19% of all Swiss wheat imports came from Canada (Schulze *et al.*, 2015). Schulze *et al*. (2015) analysed samples from the mechanical cleaning of Canadian wheat imported into Switzerland and showed the presence of 0.005% by mass of oilseed rape seeds containing the GT73 (9 samples/10) or Ms8 and Rf3 (10 samples/10) transgenes. The authors concluded that the low level of presence of oilseed rape seeds in wheat imported from Canada was the main source of dispersal of transgenic oilseed rape in Switzerland.

These three case studies from the literature confirm that "accidental" dispersal of seeds during road or rail transport or unloading from ships is common and can involve large quantities of seeds. These cases suggest that feral populations from these dispersals only persist in the event of repeated accidental dispersals or specific selective advantage (conferred by herbicide resistance). However, there was a high degree of spatio-temporal variability in all the studies, suggesting that each context is unique. In general, the persistence of these populations is

higher in agricultural fields (regrowth populations), lower on roadsides within agricultural landscapes, and lowest in the most built-up environments (railways, paved roadsides).

3.3.5. WG conclusions on the environmental risk assessment

In its technical report "*Monitoring of spontaneous populations of genetically modified plant species in the environment*", the EPA Network[31] described the different factors associated with a potentially high risk to the environment, depending on the biological characteristics of the species under consideration. Thus, the likelihood of damage occurring increases:

- with the degree to which the GM plant (GMP) tends to grow in the wild (high germination rate, long seed viability, no particular site requirements);
- with the frequency with which the GMP tends to not only grow temporarily but form self-perpetuating feral populations from spontaneously occurring plants (according to its seed dispersal capabilities);
- with the degree to which the plant in its GM-free variant already shows signs of uncontrolled growth and spread (e.g. as a weed or invasive neophyte);
- if spontaneous populations are found adjacent to conventional crops of the same plant species;
- if the local flora contains wild species capable of hybridisation in the vicinity of spontaneous GMP populations.

Oilseed rape fulfils all these risk factors. In addition, spontaneous populations of GM oilseed rape have already been reported in several countries: Switzerland and Japan but also Canada, USA, Australia and New Zealand. As in the case considered in this formal request, the cases observed in Switzerland and Japan were related to unintentional dispersal of imported seeds during unloading at port areas and/or during transport by road. However, the case studies show that each context is different. The factors to be taken into account in characterising a given situation include the following:

- The time of year when seed dispersal occurs, its recurrence and frequency

In the context of this formal request, considering:

- the variability of the spring oilseed rape cycle, which can extend from 3 to 6 months;
- the ability of seeds to germinate at several periods (mainly in spring and late summer, but potentially all year round);
- the possibility that the seeds may survive for several years due to the phenomenon of secondary dormancy;
- the fact that the GM oilseed rape plants observed in the spring of 2022 may not only be first-generation plants resulting from the germination of accidentally dispersed seeds, but also plants from later generations after reproduction;

the ANSES WG on Biotechnology concludes that it is not possible to determine the date of the event that led to this release of GM oilseed rape seeds.

[31] European Network of the Heads of Environment Protection Agencies

- The type of habitat in which the seeds are dispersed and the disturbances that may destroy or bury the seeds

In the context of this formal request, the ANSES WG on Biotechnology believes that, depending on soil disturbance and climatic conditions that are favourable or unfavourable to germination, the seeds could alternate cycles of dormancy and emergence from dormancy. New GM oilseed rape plants could therefore be observed for several years.

- The possible selective advantage conferred by the transgenes present and the selection pressure exerted

In the context of this formal request, the WG on Biotechnology considers that in the absence of any glyphosate or glufosinate-ammonium herbicide treatment, the released GM oilseed rape will not have any selective advantage at the sites examined. However, the WG recommends that the competent authorities ensure that the commitments made by the SNCF Réseau Group to stop using glyphosate are properly implemented on the railways in the Rouen industrial port area. Indeed, glyphosate herbicide exposure of the identified GM oilseed rape plants located in the surrounding area, in particular on the areas of plant cover between the road and the railway line, could induce a selection pressure in their favour, thereby promoting their persistence in the environment.

- The presence of plants capable of hybridising with GM oilseed rape plants

In the context of this formal request, the ANSES WG on Biotechnology considers that as this release only concerned plants spread over very small areas, and not a GM oilseed rape crop, any gene flow via pollen towards local, non-GM and very dominant crops could only lead to an extremely low contamination rate.

Furthermore, the ANSES WG on Biotechnology points out that according to the available data[32], there is no oilseed production in the Normandy region. The risk of seed contamination by GM oilseed rape due to the accidental release of these seeds is therefore considered to be negligible.

Among the species related to oilseed rape, considering their hybridisation rates with *B. napus* as reported in the literature and the exposure context, the ANSES WG on Biotechnology considers that the highest hybridisation risk with GM *B. napus* plants would come from wild forms of *B. rapa*.

Considering all these factors relating to exposure and environmental damage that may be associated with this "accidental" release of GM oilseed rape, the ANSES WG on Biotechnology believes that management measures, designed to eradicate the oilseed rape plants and perform monitoring, should be implemented.

[32] According to data found on the SEMAE website on accepted areas under seed production – 2021 harvest: https://www.semae.fr/etudes-donnees-statistiques-semences/

It should be remembered that the oilseed rape containing the transformation events detected during this release is authorised under Regulation (EC) No 1829/2003, and these risk factors were taken into account when granting marketing authorisation. These risk factors are partly covered by the environmental monitoring plans accompanying the authorisations, which require measures to be put in place to limit losses and spillage of viable oilseed rape and to systematically eradicate the populations found on the operators' premises.

The ANSES WG on Biotechnology considers that the measures in the monitoring plan only partially cover the risks associated with the release of GM oilseed rape into the environment of processing facilities. It is however important to ensure that these measures are properly implemented by the various operators. The WG calls for the eradication and therefore monitoring measures provided for in the monitoring plan to be extended to areas outside these facilities.

3.4. WG recommendations on managing the risks associated with the "accidental" release of GM oilseed rape into the environment

This section aims to respond to the various requests made by the DGAL in its formal request letter (Annex 1). The recommendations of the WG on Biotechnology are based on the risk assessment data presented and analysed above (Section 3.3.).

The emergency measures taken by the DGAL following the identification of GM oilseed rape plants in the environment were as follows:

- mechanical eradication of oilseed rape plants: monthly mowing and brush clearing;
- monitoring in subsequent years to ensure the absence of any regrowth that may result from the germination of seeds in the soil.

3.4.1. Measures relating to the eradication of identified GM oilseed rape plants

Considering that:

- oilseed rape plants are able to grow back from the basal rosette and that mowing or brush-clearing operations only "cut" the plant at the base of its stem without uprooting it;

- GM oilseed rape seeds may be present on the ground where the mowing or brush clearing is taking place, which could cause them to be buried and enter secondary dormancy or, conversely, cause seeds that have already been buried in the soil to emerge from secondary dormancy;

- mowing or brush clearing can also lead to seed dispersal from the mowed plants;

- the GM oilseed rape seeds imported by Saipol come from spring varieties with a flowering period of about two months;

the ANSES WG on Biotechnology concludes that eradication of the plants by mowing or mechanical brush clearing is not appropriate. The WG on Biotechnology calls for the implementation of manual uprooting, or any other method allowing complete destruction of the plants and their roots, at regular intervals to pre-empt the flowering of these plants.

In addition, the WG recommends looking for and removing any feral oilseed rape plants and plants resembling oilseed rape within a 10 kilometre radius of GM oilseed rape entry ports.

The WG reiterates that the management of eradication waste should ensure that there is no risk of release and germination of GM seeds. This entails taking the waste to the nearest treatment centre. All the trailers and dump trucks used should be covered with tarpaulins and cleaned after use (residues swept up and then washed with water, on smooth ground, i.e. without plant cover). Any parts of the freight vehicle that could potentially come into contact with the seeds, such as the tyres, should also be cleaned.

3.4.2. Additional measures to be implemented in order to avoid any persistence of GM oilseed rape

As part of the marketing authorisations for GM oilseed rape in the European Union granted under Regulation (EC) No 1829/2003, a monitoring plan must be implemented in order to identify the occurrence of any adverse effects.

This plan requires organisations to remind companies, the Saipol manufacturing facility in the case of this formal request, on an annual basis, that *"in the framework of their management or safety standards (ISO, HACCP, etc.), procedures must be in place and implemented to limit loss and spillage of viable oilseed rape and to routinely eradicate adventitious populations on their premises – any such adventitious populations, resisting routine eradication procedures, shall be treated as a potential adverse effect*". Companies must also "*report back any adverse effects reported to them to the European trade associations, including for inland transportation*".

Considering that the Saipol facility uses many different service providers and operators for:

- unloading import ships (two port terminals);
- barrowing oilseed rape seeds;
- providing freight drivers;
- monitoring seed handling, unloading and barrowing operations and taking samples from ships;
- maintaining green spaces;
- cleaning and weeding the Saipol site;
- silos.

Considering furthermore that the public establishment Haropa Port is responsible for maintaining the port area and that a close collaboration with the Saipol facility is thus expected:

the ANSES WG on Biotechnology believes that this large number of stakeholders makes it more difficult to manage and verify compliance with the implementation of procedures designed to limit loss and spillage of viable oilseed rape and to eradicate any plants, particularly around the Saipol site. The WG recommends close coordination between these different players to ensure that the procedures for limiting loss and spillage of viable oilseed rape are fully implemented, thus complying with the monitoring plan provided for in the GM oilseed rape authorisation decisions.

Considering that Saipol uses the following procedures for barrowing and securing dump truck transport between Port Terminal 2 and the silo operator:

- loading of dump trucks limited to 80%;
- checking of the dump truck door seals to ensure they are leakproof, and replacement at least once a year;
- cleaning of dump trucks before and after use (sweeping and washing with water to remove residues from the previous compartment load);
- covering of dump trucks with tarpaulins during the outward and return journeys;
- use of a limited number of trained drivers.

Considering that the seeds carried by vehicles are also usually trapped in the mud stuck to the vehicle tyres, or even in the tread soles of drivers' shoes:

the ANSES WG on Biotechnology recommends the implementation of procedures on protecting and cleaning the parts of freight vehicles likely to come into contact with seeds, especially the tyres, as well as on cleaning individual equipment of drivers and other workers in contact with the seeds.

Considering that Port Terminal 1, located in the direct vicinity of the Saipol facility, avoids the need to barrow seeds arriving at Port Terminal 2 by using a conveyor system between Port Terminal 1 and the silo operator;

Considering, however, that GM oilseed rape plants have been identified under the conveyor that transports the seeds between Port Terminal 1 and the silo site;

the ANSES WG on Biotechnology strongly recommends that Port Terminal 1 be used for unloading GM oilseed rape seeds, as close as possible to where they are stored in silos, in order to limit the transport and therefore the risk of release of these seeds. At this site, the WG recommends installing a covered (closed) conveyor belt system between Port Terminal 1 and the storage silos, in order to make the risk of seed dispersal negligible.

3.4.3. Measures relating to monitoring of the environment affected by the release

Considering:

- the frequency of barrowing between Port Terminal 2 and the silo operator on Saipol's behalf, and the fact that the release of GM oilseed rape seeds is most likely linked to this transport;

- the ability of spring oilseed rape seeds to germinate at several periods, mainly in spring and late summer, but potentially all year round;

- the ability of this spring oilseed rape to survive mild frost-free winters, with seed germination possible at 4°C;

- the variability of the spring oilseed rape cycle, which can extend from 3 to 6 months, and the fact that the objective of the monitoring measures is to pre-empt the flowering of the plants, in order to avoid the dispersal of pollen and then seeds when they reach the mature stage;

- the flowering period of the GM oilseed rape surveyed, which is around two months;

the ANSES WG on Biotechnology calls for monitoring of the emergence of oilseed rape plants and plants resembling oilseed rape throughout the year, including during the winter period, at regular intervals to pre-empt the flowering of the plants.

Considering that GM oilseed rape plants have also been identified under the conveyor that transports seeds between Port Terminal 1 and the silo operator, and that this conveyor therefore also appears to induce the spillage of GM oilseed rape seeds;

the ANSES WG on Biotechnology calls for monitoring to cover the entire Rouen industrial port area, as far as the commune of Grand-Couronne, and in particular the side of the road running between Port Terminal 2 and the silo operator, or even under the conveyors transporting the seeds to the silo site.

Considering that:

- areas with a higher density of oilseed rape plants were identified on the roadside between Port Terminal 2 and the Saipol site, particularly at a roundabout;

- in the context of the case study of road transport release near entry ports in Japan presented in this opinion, the areas with the highest plant densities were those with frequent hills or narrower road sections, and the vehicle acceleration and deceleration this induced could increase seed spillage by freight vehicles;

the ANSES WG on Biotechnology recommends that monitoring be particularly reinforced along bends in the road between Port Terminal 2 and the Saipol manufacturing facility.

Considering that:

- tillage on allotments near the identified oilseed rape plants, or mowing measures applied to the roadside, may have resulted in the burial of seeds;

- when seeds are buried in the soil, dormancy can allow them to survive for 10 to 15 years in agricultural environments;

- the current recommendations in Switzerland (Federal Office for the Environment, 2014) are to continue monitoring sites affected by releases for a further five to ten years, in a non-agricultural setting, even if no more GM oilseed rape plants are found on the site;

the ANSES WG on Biotechnology calls for the above-mentioned monitoring measures to be maintained:

- **for at least 10 years after imports of seed from countries growing GM oilseed rape varieties have ceased; they should then only stop if no GM plants are detected for two consecutive years;**
- **continuously, at regular intervals to pre-empt flowering, if imports of seed from countries growing GM oilseed rape varieties continue.**

Considering also that secondary seed dispersal is possible;

the ANSES WG on Biotechnology recommends extending the monitoring area for feral populations of oilseed rape and plants resembling oilseed rape to a 10 km radius around the site of Port Terminal 2 and the Saipol site. This monitoring should focus on roadsides along agricultural fields as well as roadsides that serve field entrances and are used during harvest by trucks transporting oilseed rape seeds to local cooperatives. On this extended perimeter only, inspections may be carried out twice a year, in spring and autumn.

Considering:

- that it is important to identify habitats that are favourable to the presence of oilseed rape, GM or not, in the environment;

- and that GM oilseed rape could hybridise with volunteer non-GM oilseed rape plants present or with other Brassicaceae species compatible with sexual crossing with oilseed rape (mainly *B. rapa* and *B. oleracea*), potentially present in the industrial port area, and that pollen flow is possible over a distance of at least 1 km, by large pollinating insects;

the WG on Biotechnology recommends continuing the sampling of the observed Brassicaceae, followed by a molecular analysis to characterise how the presence of transgenes evolves in subsequent years. These analyses may be carried out once a year. Sampling should consist of a maximum of 10 plants from a single geographical sampling point.

At the same time, the ANSES WG on Biotechnology advocates producing and annually updating a map showing the presence of volunteer oilseed rape plants, GM or not, or Brassicaceae resembling oilseed rape, throughout the area concerned by the "accidental" release and within a radius of at least 1 km of all the sites where GM oilseed rape has been identified. This map should contain:

- **A brief description of the site;**
- **The size of the population (number of individuals in the local population);**
- **The development stage of the plants (phenology);**
- **The distance between the site where the plants were identified and the nearest oilseed rape crops;**
- **The results of the molecular analyses carried out;**
- **Any special features of the site that help with interpretation of the results (disturbance, herbicide use).**

3.4.4. Measures relating to specific checks on products derived from crops grown near the sites where "accidental" release was detected, to ensure the absence of contamination by GM oilseed rape at a threshold of more than 0.9%, when these products are intended for food consumption

Considering that:

- the nearest agricultural fields were located 1097 m and 5 km from the sites where GM oilseed rape was identified and that these agricultural fields had a history of growing oilseed rape in 2020 or were used for growing oilseed rape in 2022;

- these distances are compatible with gene flow through pollen;

- any potential gene flow through pollen could, however, only result in an extremely low contamination rate given the differences in the number of plants between the pollen source (GM plants in the Rouen – Grand-Couronne industrial port area) and the fields cultivated with oilseed rape;

the ANSES WG on Biotechnology considers that sampling for molecular analysis in agricultural fields is not appropriate, because even if a transgene were present, the probability of detection would be very low.

Considering that:

- cultivated oilseed rape very often forms regrowth, and this could contain transgenes if it concerned seeds resulting from pollination by GM plants present in the Rouen – Grand-Couronne industrial port area;

- the cultivation history of the agricultural fields located near the area shows that the oilseed rape crop is mostly followed or preceded by a cereal crop (soft winter wheat or winter barley), in which the management of oilseed rape regrowth does not usually pose a problem (shallow tillage or herbicide treatment, for example combining glyphosate and 2,4D);

the ANSES WG on Biotechnology recommends specific communication to local agricultural stakeholders, asking them on the one hand to report any oilseed rape regrowth that has not been destroyed following weed control based on glyphosate alone, and on the other hand to proceed with the complete removal of these plants (uprooting) and make them available for sampling and analysis.

The WG on Biotechnology recommends providing information on the presence of this GM oilseed rape to farmers, amateur gardeners and beekeepers within a 10 km radius of the unloading sites, with a view to them monitoring and reporting the emergence of oilseed rape plants.

3.5. Conclusions of the Working Group on Biotechnology

The recommendations of the WG on Biotechnology on measures for managing the risks associated with the accidental release of GM oilseed rape seeds in the Rouen – Grand-Couronne industrial port area are as follows:

- **Concerning measures to eradicate the GM oilseed rape plants**

The ANSES WG on Biotechnology calls for:

- the implementation of manual uprooting, or any other method allowing complete destruction of the plants and their roots, at regular intervals to pre-empt their flowering.

The ANSES WG on Biotechnology recommends:

- searching for and removing any feral oilseed rape plants and plants resembling oilseed rape observed within a 10 km radius of GM oilseed rape entry ports;
- treating the waste resulting from this removal in a facility that ensures there is no risk of release and germination of GM seeds and, for this purpose, transporting it to the nearest treatment centre;
- covering the transport trailers and dump trucks used for this purpose with tarpaulins and cleaning them (including tyres). Cleaning should include sweeping the residues and then washing with water, on smooth ground without plant cover.

- **Concerning the additional measures to be implemented in order to avoid any persistence of GM oilseed rape in the environment**

The ANSES WG on Biotechnology recommends:

- particularly strict monitoring of the coordination between the various service providers working for the Saipol manufacturing facility in Grand-Couronne;
- implementation by the operator of instructions on protecting and cleaning the parts of freight vehicles likely to come into contact with seeds, especially the tyres, as well as on cleaning the equipment of drivers and other workers in contact with the seeds;
- prioritising the use of Port Terminal 1 for the unloading of GM oilseed rape seeds, and installing a covered conveyor belt system between this terminal and the storage silos;
- that the competent authorities ensure that the commitments made by SNCF Réseau to stop using glyphosate-based herbicide preparations are properly implemented on the railways in the Rouen industrial port area.

- **Concerning the measures relating to monitoring of the environment affected by the release**

The ANSES WG on Biotechnology calls for:

- monitoring of the emergence of oilseed rape plants and plants resembling oilseed rape throughout the year, including during the winter period, at regular intervals to pre-empt the flowering of the plants;
- the monitoring measures to be maintained:
    - for at least 10 years after imports of seed from countries growing GM oilseed rape varieties have ceased. This monitoring should then only be lifted if no GM plants are detected in the environment for two consecutive years;
    - continuously, at regular intervals to pre-empt flowering, if imports of seed from countries growing GM oilseed rape varieties continue;
- monitoring to cover the entire Rouen industrial port area, as far as the commune of Grand-Couronne, and in particular the side of the road running between Port Terminal 2 and the silo operator, or even under the conveyors transporting the seeds to the silo site.

The ANSES WG on Biotechnology recommends:

- reinforcing monitoring on the bends along the road between Port Terminal 2 and the Saipol site;
- extending the monitoring area for feral populations of oilseed rape and plants resembling oilseed rape to a 10 km radius around the site of Port Terminal 2 and the Saipol site. This monitoring should focus on roadsides along agricultural fields as well as roadsides that serve field entrances and are used during harvest by trucks transporting oilseed rape seeds to local cooperatives. On this extended perimeter only, inspections may be carried out twice a year, in spring and autumn;
- continuing the sampling of the observed Brassicaceae, followed by a molecular analysis to characterise how the presence of transgenes evolves in subsequent years. These analyses may be carried out once a year. Sampling should consist of a maximum of 10 plants from a single geographical sampling point.

The ANSES WG on Biotechnology advocates:

- producing and annually updating a map showing the presence of volunteer oilseed rape plants, GM or not, or Brassicaceae resembling oilseed rape, throughout the area concerned by the "accidental" release and within a radius of at least 1 km of all the sites where GM oilseed rape has been identified.

This map should contain:

- a brief description of the site;
- the size of the population (number of individuals in the local population);
- the development stage of the plants (phenology);
- the distance between the site where the plants were identified and the nearest oilseed rape crops;
- the results of the molecular analyses carried out;
- any special features of the site that help with interpretation of the results (disturbance, herbicide use).

- **Concerning the measures relating to checks on products derived from crops grown near the sites where "accidental" release was detected, to ensure the absence of contamination by GM oilseed rape at a threshold of more than 0.9%, when these products are intended for food consumption**

The ANSES WG on Biotechnology considers that sampling for molecular analysis in agricultural fields is not appropriate, because even if a transgene were present, the probability of detection would be very low.

The ANSES WG on Biotechnology recommends specific communication to local agricultural stakeholders, asking them on the one hand to report any oilseed rape regrowth that has not been destroyed following weed control based on glyphosate alone, and on the other hand to proceed with the complete removal of these plants (uprooting) and make them available for sampling and molecular analysis.

The ANSES WG on Biotechnology recommends providing information on the presence of this GM oilseed rape to farmers, amateur gardeners and beekeepers within a 10 km radius of the unloading sites, with a view to them monitoring and reporting the emergence of oilseed rape plants.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) endorses the recommendations and conclusions of the WG on Biotechnology.

ANSES points out that France has other sites for importing, storing and crushing GM seeds, or seeds coming from countries where the cultivation of GMOs is authorised. ANSES therefore recommends identifying the areas where the risk of accidental release of GM seeds is highest, which will include the following:

- (A) entry ports for GM seeds, or seeds from countries where the cultivation of GMOs is authorised;
- (B) industrial sites where GM seeds are stored or crushed;
- railways, waterways and roads used to transport goods between points (A) and (B).

ANSES stresses the need to strengthen the post-market environmental monitoring (PMEM) plans associated with the marketing authorisations for GM oilseed rape, to make them more precise and rigorous with regard to measures relating to the conditions of transport, unloading, storage and handling of these GM seeds.

The shortcomings of the monitoring plans proposed in the authorisation decisions relate to the monitoring of areas outside the GM seed processing sites. All unloading and transport areas and their immediate environment should be included in the monitoring plans to be implemented by GMO marketing authorisation holders.

The Agency recommends producing a map of these different hotspots, specifying their geographical location, and including a mechanism for annual updating. These hotspots will need to be adequately monitored, taking into account the recommendations set out in this opinion, in particular:

- monitoring at regular intervals throughout the year to pre-empt the flowering of the plants of the observed species;
- monitoring within a 10 km radius of these hotspots;
- sampling of plants observed in the monitoring areas, followed by molecular analysis, at least once a year.

The Agency considers that the European Commission could coordinate monitoring of the effectiveness of these new measures as part of the post-MA monitoring of these products.

KEY WORDS

OGM, colza, *Brassica napus*, Ms8, Rf3, GT73, MON88302, dissémination, mesures de gestion, éradication, surveillance, risque environnemental.

GMO, oilseed rape, *Brassica napus*, Ms8, Rf3, GT73, MON88302, release, management measures, eradication, monitoring, environmental risk.

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ANNEX 1 FORMAL REQUEST LETTER FROM THE DGAL

MINISTÈRE
DE L'AGRICULTURE
ET DE LA SOUVERAINETÉ
ALIMENTAIRE
Liberté
Égalité
Fraternité

Direction générale de l'alimentation

Paris, le 25 mai 2022

Le Directeur général de l'alimentation

à

Monsieur le Directeur général de l'Agence
nationale de sécurité sanitaire de l'alimentation,
de l'environnement et du travail
14 rue Pierre et Marie Curie
94701 MAISONS-ALFORT CEDEX

Objet : Saisine relative aux mesures de gestion faisant suite à une dissémination « accidentelle » d'OGM dans l'environnement

Éléments de contexte :

La présence de colza génétiquement modifié (GM) en bordure de route dans la zone portuaire de Rouen a été signalée par l'association Inf'OGM et confirmée par les analyses réalisées par le laboratoire BioGEVES sur des prélèvements effectués par les services de contrôle le long de la route entre un terminal portuaire et une usine de trituration et sur le site de l'usine. Le point du prélèvement mentionné par Inf'OGM est localisé sur le bord de la route entre le terminal portuaire et l'usine de trituration.

Ces présences d'OGM, situées à proximité et sur le site d'une usine de trituration de semences qui importe du colza GM depuis 2016, sont probablement liées à des échappements de graines lors du transport. Elles s'étendent sur une zone de plusieurs kilomètres. Les colzas étaient en fleur au moment des prélèvements.

Les OGM détectés, dans les échantillons prélevés par Inf'OGM ou par les services de contrôle, sont les colzas Ms8 et Rf3, tolérants à l'herbicide glufosinate ammonium, et les colzas GT73 et MON88302, tolérants au glyphosate.

L'utilisation de ces OGM est autorisée en alimentation humaine et animale au titre du règlement 1829/2003. Aucune autorisation n'a été délivrée pour leur mise en culture qui est de ce fait interdite dans l'Union européenne.

Dans ce contexte, une destruction des plantes par voie mécanique (fauchage, débroussaillage) a été immédiatement demandée à l'établissement public HAROPA Port en charge de l'entretien de la zone

78 rue de Varenne, 75349 PARIS 07 SP
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portuaire. Il est prévu de réitérer cette opération tous les mois, au moins jusqu'en juillet pour éviter que des repousses éventuelles ne fleurissent et ne produisent de semences qui pourraient germer à terme. Bien que le colza soit une plante annuelle, une surveillance sera mise en place les années suivantes pour vérifier l'absence de repousse qui pourraient résulter de la germination de graines déjà présentes dans le sol.

Compte tenu de la nature de la personne morale en charge de l'entretien de la zone portuaire correspondante, et conformément à l'article L. 253-7 du code rural et de la pêche maritime, l'utilisation de désherbants chimiques est proscrite.

Par ailleurs, les quelques plantes de colza observées dans l'enceinte de l'usine ont été arrachées par l'entreprise après l'inspection par les services de contrôle.

Questions :

L'objectif escompté est d'éviter toutes productions /dissémination de pollen et de graines par ces plants de colza GM et d'assurer leur destruction, leur culture étant interdite. Dans ce contexte, il est demandé à l'ANSES :

i) D'évaluer l'adéquation des mesures prescrites au regard de cet objectif ;
ii) Le cas échéant, de recenser les mesures complémentaires permettant d'éviter toute persistance de ces colzas GM sur les sites où ils ont été détectés. Dans ce cas, l'ANSES précisera la fréquence et la durée des mesures à appliquer ;
iii) De formuler des recommandations sur les mesures de surveillance adaptées (en nature, périodicité et durée dans le temps) pour vérifier l'atteinte de l'objectif.

Délai de réponse :

Nous vous saurions gré de bien vouloir nous adresser ces éléments pour le 15 septembre 2022.

Mes services se tiennent à votre disposition pour vous apporter toute information complémentaire.

Nous vous remercions de bien vouloir accuser réception de la présente demande.

Bruno FERREIRA

BRUNO FERREIRA ID

Signature numérique de BRUNO FERREIRA ID
Date : 2022.05.25 16:57:36 +02'00'

Directeur général de l'alimentation

ANNEX 2: LETTER REQUESTING FURTHER INFORMATION FROM THE DGAL

RÉPUBLIQUE FRANÇAISE
Liberté
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anses

Direction de l'évaluation
des risques

Maisons-Alfort, le 08 juillet 2022

Objet : Demande d'informations complémentaires sur la saisine n°2022-SA-0101

L'Agence nationale chargée de la sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses) a été saisie le 25 mai 2022 d'une demande d'avis relatif aux mesures de gestion faisant suite à une dissémination « accidentelle » d'OGM dans l'environnement.

Après analyse de la demande et des données disponibles pour l'expertise scientifique de ce dossier, le Groupe de travail « Biotechnologie » a identifié des éléments d'informations complémentaires qui seraient nécessaires pour mener à bien ses travaux d'expertise.

Concernant les méthodes d'échantillonnage des plants de colza génétiquement modifiés

Concernant les prélèvements effectués par les services de contrôle du ministère chargé de l'agriculture sur les lieux mentionnés par l'Association Inf'OGM (en bordure de route près de Rouen entre le terminal portuaire et l'usine de trituration de graines oléagineuses) et sur plusieurs kilomètres en bord de route (sur le trajet emprunté par les camions pour le transport de graines), ainsi que dans l'enceinte de l'usine et aux abords, le GT « Biotechnologie » souhaiterait disposer des informations suivantes :

- Une description de la méthode d'échantillonnage utilisée pour le prélèvement et l'analyse des plants de colzas ;
- Un relevé exact (points GPS par exemple) de la localisation géographique de chacun des plants de colza ayant été prélevé pour la constitution de ces échantillons, et notamment des plants qui se sont révélés positifs, afin de pouvoir, entre autres, analyser précisément la dissémination géographique des graines de colza génétiquement modifiées ;

AGENCE NATIONALE DE SÉCURITÉ SANITAIRE
de l'alimentation, de l'environnement et du travail
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- Pouvoir disposer d'un comptage précis et de la localisation géographique exacte (points GPS par exemple) des plants de colzas dont la nature transgénique serait suspectée (plants observés mais non prélevés, si l'échantillonnage réalisé pour les analyses n'était pas exhaustif).

Concernant les méthodes de détection des plants de colza génétiquement modifiés

Les éléments de contexte de la saisine précisent que les plants de colza détectés dans les échantillons prélevés par Inf'OGM ou par les services de contrôle sont les colzas Ms8 et Rf3, tolérants à l'herbicide glufosinate-ammonium, et les colzas GT73 et MON88302, tolérants au glyphosate. L'article du site internet de l'Association Inf'OGM intitulé « Agrocarburants : des colzas transgéniques aux portes de Rouen », indique que le colza transgénique détecté est le Ms8xRf3.
Dans ce contexte, le GT « Biotechnologie » souhaiterait disposer du protocole précisant les méthodes de détection qui ont été utilisées par le laboratoire BioGEVES et notamment si les analyses ont été effectuées sur plants individuels ou en mélange, et disposer d'une copie des résultats obtenus.

Concernant la nature des graines importées par l'usine de trituration de semences et les procédures de gestion des risques mises en place dans le cadre de ces imports par cette dernière

Le GT « Biotechnologie » souhaiterait disposer d'informations sur la nature exacte (caractérisation du ou des transgène(s)) et la quantité (en tonnes) de l'ensemble des graines de colza génétiquement modifiées ou non que l'usine importe depuis 2016.

Le GT « Biotechnologie » souhaiterait disposer des procédures mises en œuvre par l'usine dans le cadre de l'import de ces produits, conformément aux obligations légales décrites dans les plans de surveillance des effets sur l'environnement liés aux décisions d'autorisation de mise sur le marché des colzas génétiquement modifiés, dans l'objectif :

- de limiter les pertes et les déversements de graines viables, notamment lors du chargement et déchargement de ces dernières y compris lors du transport,
- d'éradiquer les plants présents dans ses locaux.

De plus, si l'usine importe des graines de colza "conventionnelles" (non GM) en provenance de pays où la culture de colzas génétiquement modifiés est autorisée, le GT « Biotechnologie » souhaiterait disposer également des procédures spécifiques mises en œuvre pour éviter la dispersion de ces graines lors du transport.

Concernant l'environnement du site de dissémination

Des parcelles agricoles ont pu être identifiées à proximité géographique du site de l'usine et de la zone portuaire de Rouen concernés par cette dissémination « accidentelle » de colzas génétiquement modifiés.
Le GT « Biotechnologie » souhaiterait connaître la nature de ces cultures avoisinantes (incluant les rotations culturales).

Concernant les camions en charge du transport des graines entre les terminaux portuaires de Rouen et l'usine de triturations de semences, le GT « Biotechnologie » souhaiterait disposer des informations suivantes :

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- les conditions de transport des graines : période et fréquence des transferts par camion entre le port et l'usine, type de remorques utilisées et toutes autres informations utiles à l'évaluation des risques de dissémination liés à ces transferts ;
- les procédures mises en œuvre pour leur nettoyage (sur le site de l'usine ou chez le transporteur) ; si le nettoyage n'est pas effectué sur le site de l'usine, le devenir des camions après avoir effectué le trajet décrit jusqu'à l'usine. Notamment, si ces camions appartiennent à une société de transport, des plants de colza ont-ils été recherchés/retrouvés au niveau du site du transporteur, sur la route empruntée par les camions jusqu'à ce même site ou sur tout autre trajet emprunté par les mêmes camions ?

Par ailleurs, vous indiquez dans votre courrier de saisine que des mesures de gestion sont prévues jusqu'en juillet 2022. Dans l'attente des conclusions et recommandations de l'Anses, il convient de maintenir les mesures de gestion mises en œuvre.

Je vous remercie de bien vouloir prendre en considération ces demandes et nous retourner l'ensemble des informations avant le 1er septembre 2022, compte-tenu du calendrier prévisionnel de réalisation de l'expertise indiqué dans le contrat qui vous a été adressé.

ANNEX 3

Report of the hearing with the Inf'OGM association
5 September 2022 (14:30 to 16:00)

concerning Request No. 2022-SA-0101 from the Directorate General for Food, on management measures following the "accidental" release of GMOs into the environment

Present:

Members of the Inf'OGM association

Chair and editors

Members of the ANSES Working Group (WG) on Biotechnology

Chair of the WG and expert rapporteurs

French Agency for Food, Environmental and Occupational Health & Safety (ANSES)

Scientific coordination representative from the Biotechnology Team

Head of the Biotechnology Team

Representative from the Social Sciences, Expertise & Society Unit

The Head of the Biotechnology Team opened the meeting to welcome participants and thank them for attending.

The coordination representative from the Biotechnology Team reminded participants that the Inf'OGM association was responsible for initially raising the alert about the presence of GM oilseed rape on the roadside. Following this alert, ANSES received a formal request from the Ministry of Agriculture's Directorate General for Food (DGAL).

The main objective of this hearing was to gather all the available information from the Inf'OGM association in the context of this formal request.

The coordination representative from the Biotechnology Team pointed out that a "Guide to hearings" document (attached) had been sent to all participants beforehand, and that this meeting was being recorded. She indicated that a report of the meeting would be produced and shared with all participants.

The coordination representative from the Biotechnology Team proposed a round-table discussion, with participants from Inf'OGM, the WG on Biotechnology and ANSES speaking in turn to introduce themselves.

The coordination representative from the Biotechnology Team then provided some background information on the request:

- The "accidental" release of GMOs, which was the subject of the DGAL's formal request, had been reported by the roadside in the port area of Rouen.
- It concerned GM oilseed rape plants, whose presence had initially been reported by the Inf'OGM association.
- These plants were located between the port terminal where the oilseed rape seeds arrive by ship, and the Saipol manufacturing facility, which produces crude oil and rapeseed cake.
- The DGAL had asked ANSES to assess the suitability of the measures for destroying GM oilseed rape plants, identify possible additional measures to avoid any persistence of these GM oilseed rape plants in the environment, and make recommendations on suitable monitoring measures.

The data and information collected during this hearing were to be critically analysed as part of ANSES's collective expert appraisal.

The coordination representative from the Biotechnology Team presented the agenda set out in the guide to hearings, in three parts:

I. Background information

- Presentation of the alert raised by Inf'OGM

The Inf'OGM association reported that it had spotted oilseed rape plants by chance on the side of the road in Grand-Couronne in the port suburbs of Rouen, in late February 2022 while participating in the television programme "Sur le Front", which was investigating the issue of agrofuels. While producing this news story, it had witnessed the unloading of oilseed rape seeds from a ship, with a large scoop in the open air in the port of Grand-Couronne. This unloading process resulted in seeds and dust being dispersed into the air, which is visible in its footage.

While producing this story, the Inf'OGM association was able to meet with the Saipol Group's Director of Communications, who reportedly stated that French agrofuels based on oilseed rape were partly transgenic. These GM oilseed rape imports come mainly from Australia and Canada.

Concerning the roadside sampling, Inf'OGM explained that it had been carried out randomly and without a precise protocol. Six to seven whole plants (with roots) of this oilseed rape were collected, half of which were sent to the ADGène laboratory. The other half were stored by the association in a freezer, but have since been destroyed.

The Inf'OGM association wished to point out that the ADGène laboratory currently belongs to ADM, a company it considers to be close to the oilseed rape importers.

The results of the analyses carried out by the ADGène laboratory indicated detection of the transgene corresponding to Ms8xRf3 oilseed rape (property of BASF). The analysis reports were to be sent to ANSES.

Following this, the Inf'OGM association stated that it had contacted the Ministry of Agriculture, Hugo Clément (head of the programme "Sur le Front"), and Saipol. It said it had received a response from the DGAL, which indicated it was putting in place measures to monitor the site and take samples for analysis. No other contact with the DGAL has been established since this alert.

Concerning the Saipol manufacturing facility, the Inf'OGM association said it had received a reply stating that "all possible measures have been put in place, and what you found (concerning the positive results of transgene detection) is inconsequential".

The Inf'OGM association explained that it had also sampled plants near other Saipol facilities in France: in Sète, Bassens, Mulhouse, Dieppe and Saint-Nazaire.

It also indicated that near the Saipol facility in Rouen, a new sample had been taken in July 2022, whose results were also positive. These analyses were carried out by a Eurofins laboratory.

- Discussion on the data indicated in the article entitled: *"Agrocarburants : des colzas transgéniques aux portes de Rouen"* ["Agrofuels: transgenic oilseed rape on the doorstep of Rouen"] published on the Inf'OGM website

The coordination representative from the Biotechnology Team asked the following question: "You state in your article that 'oilseed rape imports into the port of Rouen have varied between 175,000 and 550,000 tonnes per year, with an average of 300,000 tonnes', and that 'the vast majority of this oilseed rape is imported by the company Saipol'. Could you give us the sources of this data?"

The Inf'OGM association replied that it had received this information directly from the port authorities.

The coordination representative from the Biotechnology Team then asked what was meant by "the vast majority of this oilseed rape is imported by the company Saipol"; had other sources been identified?

The Inf'OGM association replied that this was the exact quote of the port official, who wished to remain anonymous. The official was unable to communicate further on what was meant by the "vast majority". The Inf'OGM association therefore chose to investigate other companies that could be concerned by oilseed rape imports, and sent questions to the DGAL, which had not yet replied. It also questioned oilseed market managers and Saipol, but did not receive any specific answer.

II. Information on the location, sampling and detection of GM oilseed rape plants

- Dates and precise locations of the sampling of oilseed rape plants carried out by Inf'OGM

The Inf'OGM association stated that the first samples in Rouen were taken in late February and the second in mid-July, on the boulevard that runs alongside the Seine in Grand-Couronne, opposite the Saipol unloading port. The second samples were not taken by Inf'OGM, but by a contact of the association, who was on the spot and was able to tell it that feral rape was growing around the Saipol manufacturing facility, that "the surroundings were regularly mown", with "some places where there is a little and others no longer any at all" and that "cutting is recent". This contact did not give any precise information on the location of the samples in Grand-Couronne, nor on the number of plants taken. Inf'OGM was to obtain further information from this person directly.

- Protocol specifying the methods for sampling and for preparation of the test samples; results of the analyses

The reports from the analytical laboratories (ADGène and Eurofins) on all the samples taken were to be sent to ANSES by Inf'OGM following the hearing.

Regarding the extent of the geographical area concerned by the release of oilseed rape, Inf'OGM indicated that the plants were not abundant. It pointed out that it had not specifically been looking for feral rape. Unfortunately, it did not have time to analyse the area more specifically. The plants it collected were all located in the same place, on the central median between the two traffic lanes, very close to the oilseed rape seed unloading port.

On the Saipol site, the Inf'OGM association indicated that some feral rape plants were growing on the railway tracks, but it was unable to access them.

Concerning the possible presence of non-transgenic oilseed rape among the samples analysed, the Inf'OGM association indicated that an overall analysis had been requested. The results indicated that "the GMO oilseed rape DNA content as determined by the detection of Ms8xRf3 from oilseed rape is 6.5%" and "the GMO oilseed rape DNA content as determined by the detection of Rf3 from oilseed rape is greater than 10%".

The Inf'OGM association indicated that it had asked Mr Yves Bertheau for an interpretation of these results. Mr Bertheau, who worked on the COEXTRA programme – GMO detection and traceability – told the association that it was not possible to determine from these reports whether all the plants were transgenic or not. Regarding the July results, this information was not yet available.

The Inf'OGM association explained that the second set of samples taken from the site in July had been entrusted to the Eurofins laboratory, replacing the ADGène laboratory, which did not wish to take on these new analyses.

The Inf'OGM association pointed out that the results from the two laboratories were not presented in the same way: ADGène presented one report per GMO, while Eurofins presented the results by target sequence detected: *bar* was detected, a gene that is found in the Ms8 and Rf3 events.

- Detection method used by the analytical laboratory consulted, in particular whether the analyses were carried out on individual plants or on mixtures

The ADGène results report (first sample from Grand-Couronne in February 2022) indicated the following analysis method: "Real-time quantitative PCR according to the requirements of the standards: ISO 24276, ISO 21569, ISO 21570, ISO 21571".

The Eurofins laboratory reports also indicated a real-time PCR method of analysis.

The Inf'OGM association added that it did not have the means to request an individual analysis of each plant.

The Inf'OGM association specified that it did not have the number of plants taken from Grand-Couronne in July 2022, but rather the weight of the sample received. It was 256.7 g (and not 856 g after checking directly on the laboratory results report), and 16 g (corresponding to seven half-plants) for the sample taken in February 2022.

III. Information relating to the environment of the release site

- Context of monitoring in France (and particularly the Rouen site) by Inf'OGM (history, current and future monitoring, etc.)

Regarding the history, the Inf'OGM association stated that these were the first samples it had taken in France. As a reminder, this sampling was unplanned and had been carried out by chance.

A campaign had been conducted by Inf'OGM for two consecutive years. The report was to be sent to ANSES. No GM oilseed rape was found. This action had been taken following the publication in 2015 by Swiss researchers of evidence of transgenic feral oilseed rape (GT73, 29 positive plants out of 136) found around the port of Basel by the biologist Mr D'Andrea (https://www.infogm.org/5138-suisse-residus-colza-OGM-importe-ble-canadien). The ships had come from Belgium or the Netherlands and travelled down to Switzerland via the Rhine. Inf'OGM had asked Mr D'Andrea to accompany them on a small sampling campaign in France, at several sites. The results were all negative.

Regarding the future monitoring of the Grand-Couronne site, the Inf'OGM association indicated that it had not yet made any decision. It stated that the association was waiting for the ANSES hearing before publishing its results on the samples taken in July 2022, and that it would then decide on the actions to be taken in this context.

On samples taken elsewhere in France:

- Sète
- Bordeaux / Bassens
- Mulhouse
- Dieppe

The results were negative in Dieppe, Bassens and Mulhouse, but there was a sampling problem in Bassens, according to Eurofins.

The Inf'OGM association described the samples it took in Sète on 23 April and 4 May 2022. In the port of Sète, the Saipol facility is located at the end of the pier, about 2 km from the town centre. An aerial photo was taken and concentric circles were drawn to visualise the distances.

The Inf'OGM association said it took samples and photos near the site on the port.

The samples were taken at the entrance to the Saipol facility, on the lane leading from the entrance gate to the port near a roundabout (within the plant site), and to the east of this roundabout on the Avenue de la Méditerranée. It took six or seven plants, half of which were sent to the Eurofins laboratory (about 80 g). The results were positive for the *Ara/Bar* gene (Ms8/Rf3). The analysis report was to be sent to ANSES, and clarification on the detection methodology was requested by Inf'OGM.

The Inf'OGM association indicated that there may be truck traffic on the site's lanes but, unlike in Rouen, the oilseed rape seeds did not seem to be transferred in the open air using large scoops, but by conveyor belts in a tunnel.

The Inf'OGM association added that the most secure system seemed to be that on the Bassens site. This site uses a "sealed pipeline" from the ships to the manufacturing facility. As a reminder, the samples taken from this site by Inf'OGM were negative.

- Flowering stage of the plants observed at the different sites

In Grand-Couronne in February, the plants were in flower (quite early), no seeds.

In Grand-Couronne in July (to be confirmed), the plants were not in flower but had seeds.

In Sète and Bassens, the plants were in flower.

- Feedback after your discussion with staff from the Saipol facility

The Inf'OGM association indicated that it had had a discussion with the Director of Communications of the Saipol facility. The Director indicated by email that "concerning the protocols put in place by Saipol to avoid the release of GMO oilseed rape seeds, in the absence of specific measures, the loss of seeds, whether GMO or non-GMO, can potentially be observed during seed unloading and transfer, which is why a specific protocol has been put in place with the following measures: daily cleaning of the unloading docks and destruction of waste in facilities dedicated to the treatment of GMO materials, verification of the leakproof nature of the trucks used to transport the seeds between the unloading dock and the storage silo, limiting each truck load to 80% of its capacity to reduce the risk of losing seeds during transport, and an annual campaign to systematically destroy any oilseed rape plants observed around the docks and the plant in order to avoid any possible regrowth." The Inf'OGM association said it had not been able to verify the proper application of these measures.

The Inf'OGM association indicated that it would send additional reports to ANSES as soon as possible. It stated that the DGAL had not been informed of the results at the Sète site.

The Inf'OGM association pointed out that it would like to be able to work with public laboratories in order to be able to investigate further.

The coordination representative from the Biotechnology Team indicated that she would inform Inf'OGM of the publication of the ANSES opinion relating to this formal request.

The Inf'OGM association wished to have information on the measures taken by the DGAL following its alert.

The Head of the Biotechnology Team indicated that this request should be addressed directly to the DGAL. He reiterated the Agency's independence from the ministries and the separation of expert appraisal work from risk management. If necessary, a presentation on this expert appraisal work could be organised after its publication.

The Chair of Inf'OGM confirmed that she was waiting for a response from the DGAL.

In conclusion, the coordination representative from the Biotechnology Team and the Head of the Biotechnology Team thanked the people from Inf'OGM for the information provided during this hearing.

ANNEX 4

Data on the location of samples taken by the inspection services (DRAAF/SRAL Normandy)

Sample	Sampling date	GPS coordinates	Site description	Characteristics
27620	19/04/2022	49.37338, 1.00826	Roadside (Bd Maritime)	Flowering stage
27631	19/04/2022	49.36459, 0.99869	Roadside (Bd Maritime)	Flowering stage
27632	19/04/2022	49.36337, 0.99785	Close to Port Terminal 1, quite far from the road. Railway line nearby.	Flowering stage
27633	19/04/2022	49.35844, 0.99074	Under the conveyors (Port Terminal 1 – storage silos) crossing the railway track, opposite the entrance to the Saipol site.	Flowering stage
27634	19/04/2022	49.39323, 1.01882	Roadside (Bd Maritime), close to an allotment garden.	Flowering stage More developed oilseed rape.
27635	19/04/2022	49.40008, 1.01935	On the outer edge of a roundabout exit. Railway line nearby.	Flowering stage
27636	19/04/2022	49.4047, 1.01586	Railway line nearby.	High density. Flowering stage
27637	19/04/2022	49.40999, 1.01744	Roadside (Bd Stalingrad). Railway line nearby.	High density. Beginning of flowering.
27638	19/04/2022	49.40998, 1.01745	Roadside (Bd Stalingrad). Railway line nearby.	Beginning of flowering.
27639	19/04/2022	49.4146, 1.01999	Roadside (Bd Stalingrad).	Rosette stage.
27640	19/04/2022	49.41496, 1.01882	Sample taken at the Inf'OGM association's sampling site. Roadside (Bd Stalingrad).	Doubt about the nature of the sample, it may be another Brassicaceae. Rosette stage.
27611	29/04/2022	49.35553, 0.99005	Inside the Saipol facility site, plants observed mainly in places that are difficult to access or maintain (around equipment, kerbs, gravel areas, ATEX areas).	Presence of scattered oilseed rape regrowth at or near flowering stage.
27612	29/04/2022	49.35509, 0.99073		
27613	29/04/2022	49.415310, 1.020309	Roadside. Northern part of Boulevard Stalingrad/Boulevard du Midi.	No information

27614	29/04/2022	49.427137, 1.030871	Roadside. Northern part of Boulevard Stalingrad/Boulevard du Midi.	No information
27615	29/04/2022	49.434699, 1.047310	Roadside. Northern part of Boulevard Stalingrad/Boulevard du Midi.	No information

ANNEX 5: TRACKING OF UPDATES TO THE OPINION

Date	Page number	Changes made
13/01/2023	12	The sentence "On 13 April 2022, the association informed the Ministry of Agriculture of this result, which was immediately acted on by the competent authorities, involving the Normandy Regional Directorate for Food, Agriculture & Forestry (DRAAF), the Normandy Regional Food Service (SRAL), the Seine-Maritime Departmental Directorate for Population Protection (DDPP) and the Normandy Regional Directorate for the Economy, Employment, Labour and Solidarity (DREETS)". was replaced by "On 13 April 2022, the association informed the Ministry of Agriculture of this result, which was immediately acted on by the competent authorities, involving the Normandy Regional Directorate for Food, Agriculture & Forestry (DRAAF) and the Normandy Regional Food Service (SRAL)".
13/01/2023	13	The legend to Figure 3: "Location of samples taken by the control services (DRAAF/SRAL Normandy, DDPP76 and DREETS Normandy) in the south-western port suburb of Rouen (in red, the GM samples; in blue, the non-GM samples) (Images from DRAAF/SRAL Normandy)" was replaced by "Location of samples taken by the control services (DRAAF/SRAL Normandy) in the south-western port suburb of Rouen (in red, the GM samples; in blue, the non-GM samples) (Images from DRAAF/SRAL Normandy)"
13/01/2023	28	The sentence: "The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the negligible risk of establishment of oilseed rape MON 88302, in the absence of a glyphosate-based herbicide treatment" was replaced by: "The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions, in the absence of a glyphosate-based herbicide treatment", so as not to summarise the EFSA position, which is described in detail elsewhere.
13/01/2023	29	The sentence: "The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions on the negligible risk of establishment of oilseed rape GT73, in the absence of a glyphosate-based herbicide treatment" was replaced by: "The ANSES WG on Biotechnology believes that the updated literature data do not call into question the EFSA conclusions, in the absence of a glyphosate-based herbicide treatment", so as not to summarise the EFSA position, which is described in detail elsewhere.
13/01/2023	76	The title of Annex 4: "Data on the location of samples taken by the inspection services (DRAAF/SRAL Normandy, DDPP76 and DREETS Normandy)" was replaced by: "Data on the location of samples taken by the inspection services (DRAAF/SRAL Normandy)."