BASF
The Chemical Company

BASF Plant Science

BASF STUDY NUMBER # 201306-001

2013 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

DATA REQUIREMENT: N/A

AUTHOR:

[*name delete*]

STUDY INITIATED ON: JUNE 10, 2013

STUDY COMPLETED ON: MARCH 14, 2014

TEST FACILITIES:

AMFLORA 2010 STARCH PRODUCTION FIELDS,
CZECH REPUBLIC, AND

PERFORMING LABORATORY:

CROPDESIGN N.V., A BASF PLANT SCIENCE COMPANY GMBH,
TECHNOLOGIEPARK 21C, 9052 ZWIJNAARDE, BELGIUM

SUBMITTED BY:

BASF PLANT SCIENCE COMPANY GMBH
CARL-BOSCH-STR. 38
D-67056 LUDWIGSHAFEN
GERMANY

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

Company: BASF Plant Science Company GmbH

Company Agent: [*name delete*] Date: _____

Title: Regulatory Compliance Manager Europe

Signature: [*signature delete*]

These data are the property of BASF Plant Science Company GmbH and, as such, are considered to be confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute in any other country.

STATEMENT CONCERNING GOOD LABORATORY PRACTICES

The study described in this volume was not conducted in compliance with the OECD Principles of Good Laboratory Practice or the GLP Principles of German Chemikaliengesetz (Chemicals Act) and does not meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 150 (FIFRA)]. The data generated by BASF Plant Science Company GmbH in support of product safety comply with generally accepted scientific procedures. Record keeping is consistent with procedures used throughout the research community. This report accurately presents the raw data developed during the studies.

STUDY MANAGER:

[*name delete*] _____ _____

Date

Sales Representative in Agro CZ
BASF spol s.r.o., AGRO
Sokolovská 668/136d
186 00 Praha 8, Czech Republic

SUBMITTER AND SPONSOR REPRESENTATIVE:

[*name delete*] _____ _____

Date

Regulatory Compliance Manager Europe
BASF Plant Science Company GmbH
Carl-Bosch-Str. 38
D-67056 Ludwigshafen, Germany

CERTIFICATION OF AUTHENTICITY

We, the undersigned, hereby declare that this study was performed under our supervison according to the procedures described herein, and that this report provides a true and accurate record of the results obtained.

STUDY MANAGER:

[*name delete*] _____ _____

Date

Sales Representative in Agro CZ
BASF spol s.r.o., AGRO
Sokolovská 668/136d
186 00 Praha 8, Czech Republic

SUBMITTER AND SPONSOR REPRESENTATIVE:

[*name delete*] _____ _____

Date

Regulatory Compliance Manager Europe
BASF Plant Science Company GmbH
Carl-Bosch-Str. 38
D-67056 Ludwigshafen, Germany

STEWARDSHIP QUALITY MANAGEMENT STATEMENT

Study No.: 201306-001

Study Title: 2013 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

BASF GB Regional Quality Manager, a member of the Stewardship Quality Management team, has inspected the Study Plan, Study File, and Final Report for compliance with the requirements for the post-market environmental monitoring of amylopectin potato EH92-527-1, variety Amflora.

[*name delete*]_____ _____

Regional Quality Manager (or delegate) Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND DEFINITIONS

CFR	Code of Federal Regulations (USA)
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act (USA)
gDNA	genomic DNA
IP System	Amflora Identity Preservation System
PCR	Polymerase chain reaction
qPCR	quantitative Polymerase chain reaction

STUDY INFORMATION PAGE

BASF Study Number	201306-001
BASF REG. DOC. NO.	2014/7000463
Study Title:	2013 Monitoring for Volunteer Potatoes at 2010 Starch Potato Production Fields
Sponsor:	BASF Plant Science Company GmbH Carl-Bosch-Str. 38 D-67056 Ludwigshafen Germany
Sponsor Representative	[*name delete*]
Testing Facilities	Amflora 2010 starch production fields, Czech Republic
Study Manager	[*name delete*]
Performing Laboratory	CropDesign N.V., a BASF Plant Science Company GmbH,Technologiepark 21c, 9052 Zwijnaarde, Belgium
Analysis Request Initiator	[*name delete*]
Analysis conducted by	[*name delete*]
Study Initiation Date:	June 10, 2013
Study Completion Date:	March 24, 2014
Location of Raw Data and Report	BASF SE, Agricultural Center Limburgerhof, APD/Q Global Process & Quality Assurance GLP Archive, LI439 Speyerer Strasse 2, 67117 Limburgerhof, Germany

2013 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

SUMMARY

The amylopectin potato EH92-527-1, variety Amflora, has been genetically modified for increased amylopectin content in the tuber starch via transformation with a gene fragment encoding granule bound starch synthase (*gbss*) from potato in antisense orientation. This modification leads to the silencing of the amylose synthesizing enzyme in the potato tuber. In March 2010, Amflora was approved for commercial cultivation in the European Union and was grown for starch potato production at locations in the Czech Republic in 2010.

As part of the Amflora post-market environmental monitoring plan the purpose of this study was to evaluate the presence and persistence of Amflora volunteer plants and their frequency in the years following the Amflora cultivation for starch production.

Out of seven fields in the Czech Republic monitored in the third year following Amflora cultivation only at one field planted with Amflora 201 volunteer plants were detected. These 201 potato volunteers were confirmed as being Amflora potato plants.

INTRODUCTION

The amylopectin potato EH92-527-1, variety Amflora, has been genetically modified for increased amylopectin content in the tuber starch and was approved for commercial cultivation in the European Union in March 2010. Cultivation for starch potato production took place in the Czech Republic in 2010.

According to the Amflora post-market environmental monitoring plan the purpose of this volunteer monitoring study was to evaluate the presence and persistence of Amflora volunteer plants and their frequency in the years following the Amflora cultivation for starch production.

At harvest of potatoes always a certain portion of potato tubers remain in the field. The survival of these remaining potato tubers depends on soil management practices and

low temperature during the winter period. Tubers which survive under winter conditions can give rise to potato plants, also known as tuber-borne volunteer potatoes, in subsequent crops.

The purpose of this study was to demonstrate that amylopectin potato EH92-527-1 is comparable to conventional potatoes with regard to its competitive behavior and the capacity to survive or tolerate environmental conditions like frost. Evidence should be given that amylopectin potato EH92-527-1 does fit in the management scheme of conventional starch potatoes and that possible volunteer potatoes will be controlled effectively by the applied cultural practices.

Annex 2 of the Amflora post-market environmental monitoring plan (EU Register, 2010) outlines the number of production sites to be selected for surveillance should be 20. Therefore all Amflora starch production fields from 2010 were included in this volunteer monitoring study which relates to seven fields in the Czech Republic.

MATERIALS AND METHODS

Potato volunteer monitoring. Monitoring was performed at all fields which were cultivated for Amflora starch production in 2010. This comprised a total of seven fields in the Czech Republic (Table 1). The monitoring was conducted at two time points during the cultivation period in 2013. The method described has been adapted from field inspection procedures and has already been used successfully for monitoring releases of genetically modified plants (MacDonald and Rouan, 2000).

Per field two to four points were chosen along one side of the field and marker stakes were driven into the ground to mark these points. Points were chosen e.g. along tractor lines passing the field to facilitate walking through the field for the observer. GPS-coordinates of the points were taken and recorded. Vertical lines through the field were mapped out and the observer walked along these lines across the field (yellow and green lines in Figures 1, 4, 8, 11, 14, 17, and 20). Per mapped vertical line six to 10 plots were selected randomly to a total number of 20 observation plots per field. The individual selected plots were at least 5 m apart from each other and the six to 10 plots were distributed randomly across the field along the mapped vertical line. As recommended by Roberts-Pichette and Gillespie (1999) for each plot an area of 1 m x 1 m was measured and this square meter represented the area to be monitored

for the occurrence of potato volunteers. The number of observed volunteers within the 1 m^2 plots was recorded. The monitoring at the field code CZ02 was conducted differently. Instead of selecting 20 plots along the lines perpendicular to the base line and monitoring the occurrence of potato plants at each of the selected 20 plots, all volunteer potato plants occurring along those lines were recorded and no plots were selected. This procedure was applied for both monitoring time points in June and August, respectively.

For any volunteer potatoes found, one leaf was taken and analyzed for identity via PCR analysis. After recording the volunteer occurrence and in order to destroy the observed volunteers according to the requirements of the Amflora IP System, they were dug out of the soil and left on the field for composting.

PCR Analysis. Leaf samples from the volunteer potatoes were taken in the field, put in bags and transported to the test facility CropDesign N.V., Zwijnaarde, Belgium. At CropDesign, the leaves from the plants were divided into two samples each for analysis. These samples were analyzed individually by a validated real-time PCR measurement.

DNA Extraction. For each sample, a small piece (approximately 20 mg) of the leave was taken. Samples were frozen lower than -65°C and homogenized by grinding for 30sec at 30 Hz in a Retsch mill according to the protocol “DNA Isolation aus Blättern unter Verwendung des Wizard Magnetic 96 DNA Plant System (Promega), SOP: SG/MA 0001/2008 Version 007. The homogenized material was lysed in Lysis Buffer A and thoroughly shaken. After centrifugation, the clear supernatant was mixed with MagneSil Paramagnetic Particles, FF377X, (Wizard Magnetic 96 DNA Plant System, FF3761X). Subsequent to a 5 minute incubation step, the DNA bound to the magnetic beads was washed 3x by placing and displacing the 96 well flat-bottom plate onto a magnet. After washing and drying of the magnetic beads, genomic DNA bound on magnetic beads was eluted with Millipore water. Two independent samples were isolated and the results obtained from the duplicate samples were equivalent.

DNA Quantification. The concentration of gDNA isolated by this method is on average around 20ng/µl, and was thus not determined for each individual sample. As mostly duplex qPCR reactions were performed, the presence of a typical DNA amount in

each individual sample was confirmed by the Ct value of qPCR reaction of the endogenous reference gene. The PMA19 assay

Quantitative Real-time PCR Setup. TaqMan® assays (Applied Biosystems, Carlsbad, CA USA) were performed in a 384 well plate format on an Life Technologies QuantStudio sequence detection system (Life Technologies), using BASF Plant Science internally validated real-time qPCR TaqMan® *duplex* assays SG-Assay 0426 (Amflora),

Assay conditions and primers and probe sequences were as described in JRC (2009) using an endogenous potato gene as reference (starch branching enzyme, StSBE1).

Raw data of qPCR analysis are the threshold cycles (Ct) as the point where the instrument first detects fluorescence above the background. The Cts for both the event-specific target and the potato endogenous reference gene StSBE1 for each sample were determined. A delta Ct value (dCt), representing the difference between the Ct value for the Target assay and the Ct value for the endogenous reference gene, was calculated for each sample. Results for the presence of the Target element per event in each sample were calculated as either positive or negative based on the dCt value for each sample.

Controls. Each 384 well PCR plate included a 5-stage dilution series of the gDNA standard control, one wild type as a negative control and one well without any DNA template as a negative control for the reference endogene amplicon.

RESULTS AND DISCUSSION

A total of seven Amflora production fields in the Czech Republic were monitored for the occurrence of potato volunteers. Figures 1 to 22 illustrate the monitoring methodology as applied in 2013. During the third year following the cultivation of Amflora the monitoring was conducted in June and August 2013 within the field. The results of the observations for volunteer potatoes are summarized in Table 1. Only at one field (CZ02) a total of 201 volunteer potato plants were observed within the cultivated area (Table 1, Figures 5, 6, and 7). These plants were found at both time points in June and August. Leaf samples from 68 volunteer plants observed in June and 133 in August at the field CZ02 (Table 1) were taken for further molecular analysis, and all volunteer plants were confirmed to be derived from EH92-527-1

potatoes. It was not possible to locate the mother tuber in the soil from which the potato volunteers developed. Most likely the mother tuber was buried in deep soil.

A plausible reason for the occurrence of volunteers within the field CZ02 might be that growing conditions for potato volunteers are more favorable in a maize field than within a barley or wheat field where the emerging potato plant would get less light compared to a late developing maize field.

CONCLUSIONS

The current study provides evidence that in most case (six out of seven fields) potential potato volunteer plants have been destroyed completely by soil management practices and frosts following the Amflora cultivation in 2010. This clearly demonstrates that the persistence of Amflora tubers does not differ from any other potato variety and that the cultivation and management practices applied during and following Amflora cultivation are appropriate to manage the potential survival of Amflora tubers remaining in the soil after harvest.

Similar to the results obtained from the first two years of monitoring after Amflora cultivation (2011, 2012) potato volunteer plants were only detected in those fields where the following crop was maize. Thus it can be noticed that Amflora tubers surviving the soil treatment and frost period find more favorable conditions in a late emerging crop like maize as compared to other crops used in rotation with potato like wheat, barley or oilseed rape.

REFERENCES

EFSA (2006) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-UK-2005-14) for the placing on the market of genetically modified potato EH92-527-1 with altered starch composition, for production of starch and food/feed uses under Regulation (EC) No 1829/2003 from BASF Plant Science. EFSA Journal 324, 1-20. Available online: http://www.efsa.europa.eu/de/efsajournal/pub/324.htm

JRC (2009) Event-specific method for the quantification of amylopectin potato event EH92 527 1 using real-time PCR. Available at: http://gmo-crl.jrc.ec.europa.eu/summaries/EH92-527-Validated_method.pdf

MacDonald, R. and Rouan, D. (2000) Monitoring commercial releases of genetically modified crops: InVigor Hybrid Canola. The 6th International Symposium on The Biosafety Of Genetically Modified Organisms, July 2000, Saskatoon, Canada.

Roberts-Pichette, P. and Gillespie, L. (1999) Terrestrial vegetation biodiversity monitoring protocols. EMAN Occasional Paper Series, Report No. 9. Ecological Monitoring Coordinating Office, Burlington, Ontario.

Table 1. Number of potato volunteers in 2011, 2012 and 2013 at all fields which were cultivated for Amflora starch production in 2010

Field Code	Number of volunteers observed								
	2011			2012			2013		
	Crop	Jun	Aug	Crop	Jun	Aug	Crop	Jun	Aug
CZ01	spring barley	0	0	peas& other feed crops	0	0	mix of Trifolium &other crops	0	0
CZ02	spring barley	0	0	maize	0	2	maize	68	133
CZ03	spring barley	0	0	peas & other feed crops	0	0	mix of Trifolium &other crops	0	0
CZ04	maize	3	0	spring barley	0	0	spring barley	0	0
CZ05	spring wheat	0	0	maize	0	0	maize	0	0
CZ06	spring barley	0	0	oilseed rape	0	0	winter wheat	0	0
CZ07	spring barley	0	0	oilseed rape	0	0	winter wheat	0	0

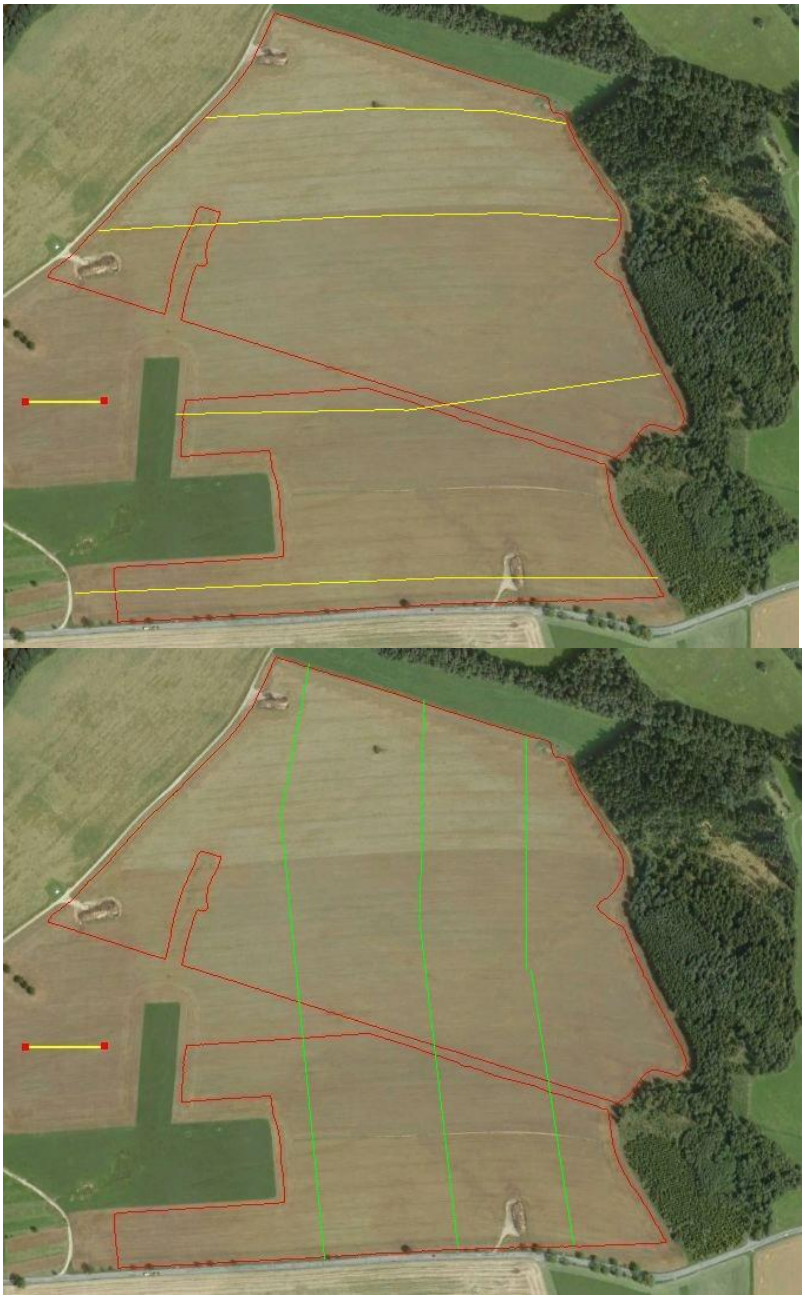

Figure 1. Fields CZ01 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the fields to select the observation plots in June and August 2013 respectively.

Figure 2. Field CZ01 – picture from volunteer monitoring

Field CZ01 and field surroundings at time of first volunteer monitoring in June 2013.

Figure 3. Field CZ01 – picture from volunteer monitoring

Field CZ01 at time of second volunteer monitoring in August 2013 conducted after harvest of the cultivated feed crops.

Figure 4. Field CZ02 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the field. All volunteer potato plants occurring along those lines were recorded in June and August 2013 respectively.

Figure 5. Field CZ02 – picture from volunteer monitoring
Field CZ02 at time of first volunteer monitoring in June 2013.

Figure 6. Field CZ02 – picture from volunteer monitoring
Field CZ02 at time of second volunteer monitoring in August 2013.

Figure 7. Field CZ02 – picture from volunteer monitoring
Potato volunteer plant within the field CZ02 in August 2013.

Figure 8. Field CZ03 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the field to select the observation plots in June and August 2013 respectively.

Figure 9. Field CZ03 – picture from volunteer monitoring
Field CZ03 at time of first volunteer monitoring in June 2013.

Figure 10. Field CZ03 – picture from volunteer monitoring
Field CZ03 at time of second volunteer monitoring in August 2013 conducted after harvest of the cultivated feed crops.

Figure 11. Field CZ04 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the field to select the observation plots in June and August 2013 respectively.

Figure 12. Field CZ04 – picture from volunteer monitoring
Field CZ04 at time of first volunteer monitoring in June 2013.

Figure 13. Field CZ04 – picture from volunteer monitoring
Field CZ04 at time of second volunteer monitoring in August 2013 conducted after harvest of barley.

Figure 14. Field CZ05 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow and green lines indicate the paths taken through the field to select the observation plots in June and August 2013 respectively.

Figure 15. Field CZ05 – picture from volunteer monitoring
Field CZ05 at time of first volunteer monitoring in June 2013.

Figure 16. Field CZ05 – picture from volunteer monitoring
Field CZ05 at time of second volunteer monitoring in August 2013.

Figure 17. Field CZ06 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the field to select the observation plots in June and August 2013 respectively.

Figure 18. Field CZ06 – picture from volunteer monitoring

Field CZ06 and field surroundings at time of first volunteer monitoring in June 2013.

Figure 19. Field CZ06 – picture from volunteer monitoring

Field CZ06 at time of second volunteer monitoring in August 2013 conducted after harvest of wheat

Figure 20. Field CZ07 – paths for monitoring

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; yellow (upper) and green (lower) lines indicate the paths taken through the field to select the observation plots in June and August 2013 respectively.

Figure 21. Field CZ07 – picture from volunteer monitoring

Field CZ07 and field surroundings at time of first volunteer monitoring in June 2013.

Figure 22. Field CZ07 – picture from volunteer monitoring

Field CZ07 at time of second volunteer monitoring in August 2013 conducted after harvest of wheat.