

ANNEX 11

GLYCOALKALOID LEVELS IN TUBERS OF AMFLORA STARCH POTATOES GROWN IN 2010

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**GLYCOALKALOID LEVELS IN TUBERS OF AMFLORA
STARCH POTATOES GROWN IN 2010**

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PAGE 1 of 12

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TABLE OF CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS	2
STATEMENT OF COMPLIANCE	3
TABLE OF CONTENTS	4
LIST OF TABLES.....	5
ABBREVIATIONS AND DEFINITIONS.....	5
SUMMARY.....	6
INTRODUCTION	6
MATERIALS AND METHODS	7
RESULTS AND DISCUSSION	8
CONCLUSIONS.....	8
REFERENCES	9
APPENDIX.....	12

LIST OF TABLES

Table 1. Glycoalkaloid Content of Pooled Tuber Samples Harvested in the Czech Republic in 2010 (in mg/kg fw)	10
Table 2. Total Glycoalkaloid Levels in Tubers Harvested in 2010 (in mg/kg fw)	11
Appendix Table A. Total Glycoalkaloid Levels in Tubers Harvested in 1996, 1997 and 1998 (in mg/kg fw)	12

ABBREVIATIONS AND DEFINITIONS

CFR	Code of Federal Regulations (USA)
CZ	Czech Republic
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act (USA)
<i>gbss</i>	Granule bound starch synthase gene
fw	Fresh weight
Max	Maximum
Min	Minimum
Rep	Replicate
RP-HPLC	Reverse phase high-performance Liquid Chromatography

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SUMMARY

The potato variety Amflora (event EH92-527-1) 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 production at locations in the Czech Republic in 2010.

Glycoalkaloids are the main toxins in potato, with the most common glycoalkaloids are α -solanine and α -chaconine (Friedmann, 2006). As part of the Amflora post-market environmental monitoring plan (EU Register, 2010), the purpose of this study was to determine the levels of glycoalkaloids in Amflora starch potatoes grown at field locations in 2010. Tubers were sampled after harvest from seven locations in the Czech Republic. A total of 28 pooled Amflora samples were analysed for their glycoalkaloid content by RP-HPLC. An additional eight pooled samples from the conventional potato variety Bonanza serving as a control were also analysed. All Amflora total glycoalkaloid measurements from 2010 were well in the range of levels measured for Amflora in 1996 to 1998 (Notification C/SE/96/3501 according to Directive 2001/18/EC). These results confirm that the glycoalkaloid content of Amflora is stable over time and remains comparable to conventional starch potato varieties, and thus verify the assumption made in the environmental risk assessment as contained in notification C/SE/96/3501.

INTRODUCTION

The potato line EH92-527-1 (OECD Unique Identifier BPS-25271-9) has been genetically modified for increased amylopectin content in the tuber starch. The mother starch potato variety Prevalent was transformed with a construct containing a gene fragment encoding granule bound starch synthase (*gbss*) from potato in reverse (antisense) orientation under the control of the potato *gbss* promoter. A kanamycin resistance gene from *Escherichia coli* under the control of

the nopaline synthase promoter from *Agrobacterium tumefaciens* allowed selection of the transformant in tissue culture. The potato line EH92-527-1 with the variety name Amflora was approved for commercial cultivation in the European Union in March 2010 and was cultivated for starch production in the Czech Republic in 2010.

The purpose of this study was to determine the levels of glycoalkaloids in Amflora starch potatoes grown at field locations in the Czech Republic in 2010, in order to confirm that the glycoalkaloid content of Amflora is stable over time and remains comparable to conventional starch potato varieties. As outlined in the post-market environmental monitoring plan for Amflora (EU Register, 2010), tubers were sampled and pooled after harvest from seven locations in the Czech Republic in 2010. The pooled samples were then shipped to TNO (Zeist, The Netherlands) for determination of their glycoalkaloid content.

MATERIALS AND METHODS

Source of Plant Material. Amflora potatoes were cultivated for starch production at 7 field locations in the Czech Republic in 2010 (Table 1). The tuber sampling followed the outline provided in the post-market environmental monitoring plan for EH92-527-1 potato (EU Register, 2010), which calls for sampling after harvest at the starch potato cultivation sites in four replicates. At each of the seven field location, four pooled tuber samples were collected, each consisting of 10 individual tubers. A total of 28 Amflora pooled tuber samples were prepared in this way. In addition, four pooled tuber samples, each consisting of 10 individual tubers, were taken from the conventional starch potato variety Bonanza grown in both Kristianstad in Sweden and Baalberge in Germany. These eight pooled samples served as the control samples for the analysis.

In total 36 pooled samples were shipped to TNO in The Netherlands at ambient temperature for analysis of glycoalkaloid content.

Glycoalkaloid Determination. Glycoalkaloid analysis was performed with RP-HPLC according to Houben and Brunt (1994). Briefly, the glycoalkaloids were extracted from the potato sample by ion-pair extraction on Sep-Pak C₁₈ columns (Waters, Milford, MA). The samples were separated and analysed on Nucleosil 5 C₁₈ AB columns using acetonitrile-water (60:40) as

eluent. The levels of the glycoalkaloids α -solanine, α -chaconine and β -chaconine were measured. The limit of detection of the total glycoalkaloid content was 2 mg glycoalkaloid per 100 gram.

RESULTS AND DISCUSSION

Glycoalkaloid composition was determined for samples of Amflora and Bonanza tubers, a conventional starch potato variety that served as control (Table 1). For Amflora, the means of total glycoalkaloid measurements from all locations was 128 mg/kg fw, with a range of 116.8 mg/kg fw at CZ05 to 134.3 mg/kg fw at location CZ04. In the case of the Bonanza tubers, means for the total glycoalkaloid levels of 92.8 and 140.2 mg/kg fw, were determined in samples from Sweden and Germany, respectively. The mean levels for total glycoalkaloids in Amflora tubers fall well within the range of measurements of the conventional Bonanza tubers.

When comparing the levels of total glycoalkaloids from Amflora tubers grown in 2010 (Table 2), to those grown in previous years (Appendix, Table A), the means of total glycoalkaloid values for Amflora in 2010, as well as the minimum and maximum values, fall well within the range of the total glycoalkaloid values for Amflora as determined in the field seasons from 1996 to 1998 and as presented in notification C/SE/96/3501 according to Directive 2001/18/EC.

CONCLUSIONS

All Amflora total glycoalkaloid measurements from 2010 were well in the range of levels measured for Amflora in 1996 to 1998 (Notification C/SE/96/3501 according to Directive 2001/18/EC) and those of the conventional starch potato variety Bonanza. These results confirm that the glycoalkaloid content of Amflora is stable over time and remains comparable to conventional starch potato varieties, and thus verify the assumption made in the environmental risk assessment as contained in notification C/SE/96/3501.

REFERENCES

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Table 1. Glycoalkaloid Content of Pooled Tuber Samples Harvested in the Czech Republic in 2010 (in mg/kg fw)

Potato Variety	Location (Field Identifier)	Rep	α -solanine	α -chaconine	β -chaconine	Total Glycoalkaloids	Mean of Total (Min-Max)
Amflora	CZ01 (630-1120-5502)	1	42	79	4	125	132.0 (116-146)
		2	43	69	4	116	
		3	47	93	7	146	
		4	52	77	12	141	
Amflora	CZ02 (630-1120-4601/1)	1	33	69	5	107	125.5 (107-170)
		2	34	69	9	112	
		3	59	94	18	170	
		4	37	67	9	113	
Amflora	CZ03 (620-1110-7703/1)	1	40	62	15	117	125.8 (117-140)
		2	46	73	10	129	
		3	40	67	10	117	
		4	50	77	13	140	
Amflora	CZ04 (620-1110-7705/6)	1	52	95	6	152	134.3 (120-150)
		2	37	76	6	120	
		3	40	75	6	121	
		4	51	91	3	144	
Amflora	CZ05 (620-1110-7705/2)	1	39	66	9	114	116.8 (103-140)
		2	35	57	11	103	
		3	48	81	12	140	
		4	38	66	6	110	
Amflora	CZ06 (650-1110-0402/11)	1	61	90	14	165	132.0 (90-165)
		2	46	64	9	119	
		3	55	80	19	154	
		4	29	55	6	90	
Amflora	CZ07 (650-1110-0402/1)	1	44	85	8	137	130.3 (116-137)
		2	39	70	8	116	
		3	48	69	17	134	
		4	45	77	12	134	
Bonanza	Kristianstad (SE)	1	42	47	5	93	92.8 (80-101)
		2	41	48	8	97	
		3	37	39	4	80	
		4	43	56	2	101	
Bonanza	Baalberge (DE)	1	86	85	7	178	142.0 (120-178)
		2	65	68	9	142	
		3	53	64	3	120	
		4	56	68	4	128	

Rep = replicate; CZ = Czech Republic; SE = Sweden; DE = Germany; Min = Minimum; Max = Maximum

Table 2. Total Glycoalkaloid Levels in Tubers Harvested in 2010 (in mg/kg fw)

2010	
	EH92-527-1
Mean	128
Maximum	170
Minimum	90
Conventional Variety	Bonanza
Mean	117
Maximum	178
Minimum	80

APPENDIX

**Table A. Total Glycoalkaloid Levels in Tubers Harvested in 1996, 1997 and 1998
(in mg/kg fw)**

Data from Notification C/SE/96/3501 according to Directive 2001/18/EC

	1996	1997	1998
	EH92-527-1		
Mean	83	209	154
Maximum	116	390	272
Minimum	61	92	104
Conventional Variety	Prevalent		
Mean	83	336	258
Maximum	122	570	436
Minimum	49	139	124