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in Annex I of Council Directive 91/414/EEC**



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Amitrole

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Table of contents

B.7. RESIDUE DATA.....	4
B.7.1. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES IN PLANTS (ANNEX IIA 6.1 AND ANNEX IIIA 8.1).....	4
B.7.1.1. Summary of metabolism previously submitted for apple	4
B.7.1.2. Additional information about the synergist: ammonium thiocyanate (a.k.a ATC).....	5
B.7.1.3. Report on olive with radiolabelled amitrole	7
B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES IN LIVESTOCK (ANNEX IIA 6.2 AND ANNEX IIIA 8.1).....	8
B.7.3. DEFINITION OF THE RESIDUE (ANNEX IIA 6.7, ANNEX IIIA 8.6)	8
B.7.3.1. Definition of the residue in plants and plant products	8
B.7.3.2. Definition of the residue in animal products	9
B.7.4. USE PATTERN	9
B.7.5. IDENTIFICATION OF CRITICAL GAPS.....	14
B.7.6. RESIDUES RESULTING FROM SUPERVISED TRIAL (ANNEX IIA 6.3, ANNEX IIIA 8.2).....	14
B.7.6.1. Sample storage stability for analysis (annex IIA 6.0).....	14
B.7.6.2. Supervised trials on orchards.....	17
B.7.6.3. Supervised trials on grapes	21
B.7.6.4. Supervised trials on olives	34
B.7.6.5. Summary on available residue data for intended uses on orchard, grape and olive	42
B.7.7. EFFECTS OF INDUSTRIAL PROCESSING AND/OR HOUSEHOLD PREPARATION (ANNEX IIA 6.5, ANNEX IIIA 8.4).....	43
B.7.8. LIVESTOCK FEEDING STUDIES (ANNEX IIA 6.4, ANNEX IIIA 8.3).....	43
B.7.9. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS (ANNEX IIA 6.6, ANNEX IIIA 8.5)	43
B.7.10. PROPOSED PRE-HARVEST INTERVALS FOR ENVISAGED USES, OR WITH HOLDING PERIODS, IN THE CASE OF POST-HARVEST USES (ANNEX IIA 6.8, ANNEX IIIA 8.7)	43
B.7.11. MRLs IN EU MEMBER STATES (ANNEX IIIA 12.2)	43
B.7.12. PROPOSED EU MRLs AND JUSTIFICATION FOR THE ACCEPTABILITY OF THOSE MRLs (ANNEX IIA 6.7, ANNEX IIIA 8.6)	43
B.7.13. ESTIMATES OF POTENTIAL AND ACTUAL DIETARY EXPOSURE THROUGH DIET AND OTHER MEANS (ANNEX IIA 6.9, ANNEX IIIA 8.8)	44
B.7.13.1. Risk assessment for chronic exposure through diet.....	44
B.7.13.2. Risk assessment for acute exposure through diet	46
B.7.14. SUMMARY AND EVALUATION OF RESIDUE BEHAVIOUR (ANNEX IIA 6.10, ANNEX IIIA 8.9).....	48
B.7.15. REFERENCES RELIED ON.....	49
B.7.15.1. New studies since first inclusion	49
B.7.15.2. References and scientific publications.....	51

List of tables

Table 7.1.1-1 : Summary of plant metabolism studies previously submitted	4
Table 7.3.1-1: Residue definition for monitoring and risk assessment proposed in plant for amitrole.....	9
Table 7.3.2-1 Residue definition for monitoring and risk assessment proposed in animal for amitrole	9
Table 7.3.2-1 : Summary of intended uses amitrole.....	10
Table 7.6.1-1 : Residues of amitrole in apple after storage in frozen condition (Biever, stability study on apple).....	14
Table 7.6.1-2 : Residues of amitrole in grape after storage in frozen condition (Clemson, stability study)	15
Table 7.6.1-3 : Residues of amitrole in grape after storage in frozen condition (Amic, stability study on grape)	16
Table 7.6.1-4 : Residues of amitrole in olive after storage in frozen condition (Amic, stability study on olives)	16
Table 7.6.2-1 : Overview of the uses of amitrole on orchards in EU	17
Table 7.6.2-2 : Summary of global information (Biever study, residue trial on apple).....	19
Table 7.6.2-3 : Details about the trials (Biever study, residue trial on apple).....	19
Table 7.6.2-4 Residues data summary from supervised trials (summary) (Biever study on apple)	20
Table 7.6.2-5 : Summary of global information (Amic study on apple)	21
Table 7.6.3-1 : Overview of the uses of amitrole on orchards in EU	21
Table 7.6.3-2 : Summary of global information (Provot study, residue trial on grape)	22
Table 7.6.3-3 : Details about the trials (Provot study, residue trial on grape).....	22
Table 7.6.3-4 : Residues data summary from supervised trials (summary) (Provot study on grapes)	23
Table 7.6.3-5 : Summary of global information (Wilson study, residue trial on grape)	24
Table 7.6.3-6: Details about the trials (Wilson study, residue trial on grape).....	24
Table 7.6.3-7 : Residues data summary from supervised trials (summary) (Wilson study on grape).....	26
Table 7.6.3-13 : Summary of global information (Amic study, residue trial on grape)	32
Table 7.6.3-14 : Details about the trials (Amic study, residue trial on grape).....	32
Table 7.6.3-15 : Residues data summary from supervised trials (summary) (Amic study on grape).....	33
Table 7.6.4-1: Overview of the uses of amitrole on olives in EU	34
Table 7.6.4-2 Summary of global information (Anonymous, residue trial on olive)	34
Table 7.6.4-3 : Summary of global information (Amic study, residue trial on olive)	35
Table 7.6.4-4 : Details about the trials (Amic study, residue trial on olive)	35
Table 7.6.4-5 : Residues data summary from supervised trials (summary) (Amic study on olive)	36
Table 7.6.4-9 : Summary of global information (Amic study, residue trial on olive).....	40
Table 7.6.4-10 : Details about the trials (Amic study, residue trial on olive)	40
Table 7.6.4-11: Residues data summary from supervised trials (summary) (Amic study on olive)	41
Table 7.6.5-1 Summary of residue results on orchard, grapes and olives	42
Table 7.13.1-1 EU scenario including MRL of intended uses with amitrole	45
Table 7.13.2-1 : EFSA model (PRIMO) for acute risk assessment - rev. 2 for amitrole	47

B.7. RESIDUE DATA**B.7.1. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES IN PLANTS (ANNEX IIA 6.1 AND ANNEX IIIA 8.1)****B.7.1.1. Summary of metabolism previously submitted for apple**

The residue behaviour and metabolism of amitrole (aka: aminotriazole) was investigated in some fruits (apple tree) using ^{14}C labelled amitrole aqueous solution and following:

- soil application (8 kg a.i./ha) under outdoor conditions
- soil application in planting containers
- contact between amitrole solution and the cut surface of excised sprouts
- mixture with parenchymatic cells suspension cultures from mature apple fruit

The characteristics of these previously submitted studies are summarized in the following table:

Table 7.1.1-1 : Summary of plant metabolism studies previously submitted

Crop	Label position	Formulation	Type of treatment (foliar, seed, ...) (F) or (G) or (I)	Application details			Ref.
				Growth stage at application	Rate (kg a.s/ha)	No	
Fruits and fruiting vegetables APPLE	mixture of $[3-^{14}\text{C}]$, $[5-^{14}\text{C}]$, $[3,5-^{14}\text{C}]$ amitrole	aqueous $[^{14}\text{C}]$ amitrole solution	Outdoor soil treatment in field (pH 7.2) with 5-7 years old apple trees (2 specimens : I and II = 1 variety : Gelber Koestlicherr)	First year (trees I & II) pre-treatment April 1985 1 st R*:28 May 85 2 nd R*:9 July 85 Second year (tree I only) 1 st R*:14 May 86 2 nd R*:9 July 86	Pre-treat. with un-radiolabelled amitrole : 666mg/m ² (eq.to 6.7kg/ha) 4 th $[^{14}\text{C}]$ amitrole treatment with 122mg/m ² (year 85) 200mg/m ² (year 86) (eq.to 1.2 and 2kg/ha) 2 nd $[^{14}\text{C}]$ amitrole treatment with 22mg/m ² (year 85) 200mg/m ² (year 86) (eq.to 0.22 and 2kg/ha)	2/ year	Mature fruits 1 st year Sept. (83 DALA) 31 apples 2 nd year Oct. (97 DALA) 114 apples Twigs from apple trees 14 May 86 (1 st applic.) and 545 DALA
	mixture of $[3-^{14}\text{C}]$, $[5-^{14}\text{C}]$, $[3,5-^{14}\text{C}]$ amitrole	aqueous $[^{14}\text{C}]$ amitrole solution	Outdoor soil treatment in planting containers with 5-7 years old apple trees (2 specimens = 2 varieties: Gelber Koestlicher (G.K.) and Gloster (G.))	Trees IV & V 1 st R*:14 May 86 2 nd R*:9 July 86	1 st $[^{14}\text{C}]$ amitrole treatment with 200mg/container 2 nd $[^{14}\text{C}]$ amitrole treatment with 200mg/container	2	Mature fruits Oct. (97 DALA) 25 apples (11 from G.K.var. and 14 from G.var.) Concurrently with the harvest the tree IV was cut into roots, trunk and twigs of different age. The leaves which were still almost completely present were also collected
	mixture of $[3-^{14}\text{C}]$, $[5-^{14}\text{C}]$, $[3,5-^{14}\text{C}]$ amitrole	aqueous $[^{14}\text{C}]$ amitrole solution	Excised sprouts (10) from apple trees (G.K. var.) placed with the cut surface in 5mL of $[^{14}\text{C}]$ amitrole aqueous solution in greenhouse condition	Contact with $[^{14}\text{C}]$ amitrole solution during 6 hours and then placed in water and allowed to stand for 19 days (June-July 85)	8.4mg $[^{14}\text{C}]$ amitrole per sprout	1	about 40cm with 1-2 years old sections, with 2-3 fruits of about 30g on each of the 2- year-old sections
	mixture of $[3-^{14}\text{C}]$, $[5-^{14}\text{C}]$, $[3,5-^{14}\text{C}]$ amitrole	sterile $[^{14}\text{C}]$ amitrole solution	Directly applied into the cells culture (cells from parenchymatic tissue taken from mature fruit after surface sterilization with NaOCl)	Cells cultures at the beginning of the stationary phase of growth	Several experiments with sterile $[^{14}\text{C}]$ amitrole solution : 2x 0.0059mg 2 x 0.059mg 2 x 0.59mg 2 x 5.9mg 22 x 2.94mg 3 x 29.4mg	1	analysis after 7 days of incubation in normal growth conditions

(a) Outdoor or field use (F), glasshouse application (G) or indoor application (I) R* : radiolabelled
DALA (days after last application)

Mature fruits from outdoor soil treatment experiments contained at most 0.05 mg/kg TRR. The highest portion of the TRR was identified as triazolyalalanine¹ (at most 0.012 mg eq./kg, 22-24%), which occurred in the free form and as conjugates. Parent amitrole itself was not detectable. Of the TRR about 75 % was soluble and 25 % was bound to insoluble material.

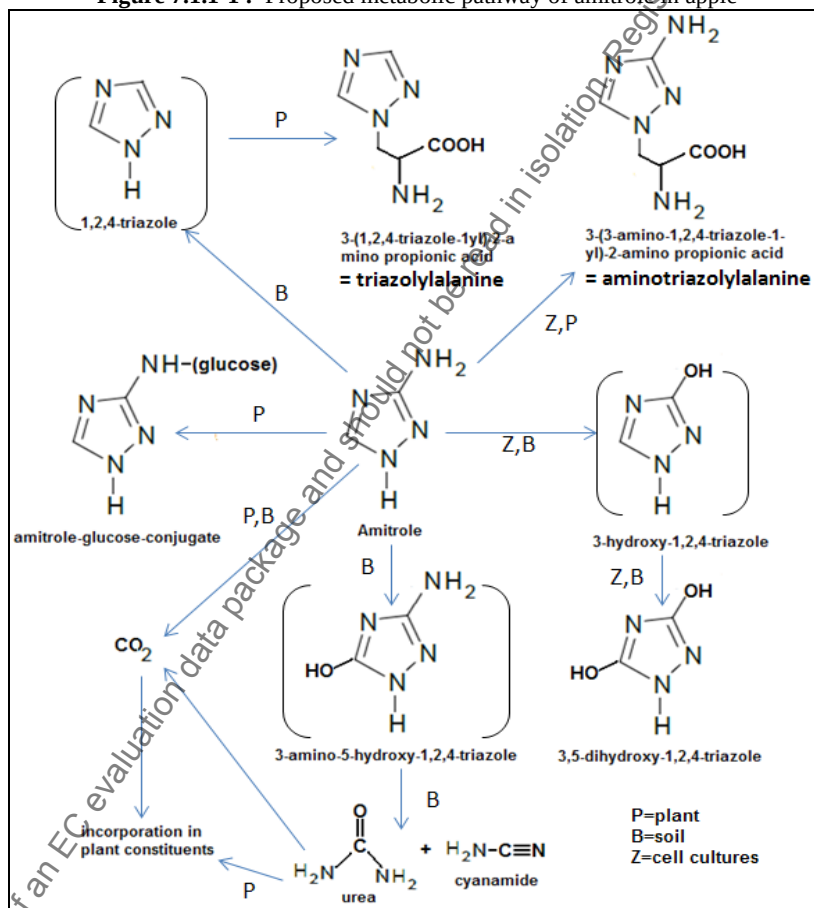
More than 50 % of the radioactivity was reassimilated ^{14}C incorporated into natural plant constituents. A part of the reassimilated ^{14}C was present within the insoluble residues and characterised after treatment with cellulase and pectinase.

In the tub experiments one apple tree absorbed 1.1% of the radioactivity applied to the soil. 0.07% was found within the mature fruits and about 42% remained in the soil (after 5 months). The loss of radioactivity of about 57% can be explained predominantly by the degradation of amitrole in the soil to $^{14}\text{CO}_2$.

In contrast to mature fruits from outdoor experiments, the major metabolite in model experiment (excised apple sprouts and cell suspension cultures) was not triazolyllalanine but aminotriazolyllalanine². In excised sprouts from apple trees small amounts of triazolyllalanine were also present. In cell suspension cultures at high concentrations of amitrole mainly 3,5-dihydroxy-1,2,4-triazole was also found.

In apple plants, amitrole appears to be metabolised to triazolyalanine presumably via the formation of aminotriazolyalanine from amitrole with serine. There is evidence, in apples, of formation of conjugates involving triazolyalanine.

Figure 7.1.1-1 : Proposed metabolic pathway of amitrole in apple



B.7.1.2. Additional information about the synergist: ammonium thiocyanate (a.k.a ATC)

The representative product CA2735 (WEEDAZOL TL) for amitrole includes the synergist ammonium thiocyanate. Since the metabolism on apple was not realized with the formulated product containing the synergist, additional information was requested to the applicant in order to estimate if the synergist would influence significantly the previously described metabolism pathway in plants and consequently would influence the residue definition.

triazolylalanine : 3-(1,2,4-triazol-1-yl)-2-aminopropionic acid

² aminotriazolylalanine : 3-(3-amino-1,2,4-triazol-1-yl)-2-aminopropionic acid

The applicant stated that: “ammonium thiocyanate acts as a synergist of amitrole and is used in most of the amitrole-based formulations and at least in CA2735 (representative formulation). In plants, the major metabolites detected are triazolyalanine and aminotriazolyalanine as a result of amitrole treatment. Triazolyalanine was reviewed by FAO/WHO in 1989 and it was concluded that residues of triazolyalanine did not present a toxicological hazard. Consequently only amitrole was regarded as residue of concern.

Nevertheless this metabolite, amitrole as well as aminotriazolyalanine were analyzed as part of recent residue trials conducted on grapes and olives. None of these compounds was detected (KIIA 6.3.3/01 and B6.3.3/02).

In fact once in plants, amitrole is conjugated to amino acids like serine to form aminotriazolyalanine³. Most of these conjugates are less toxic than aminotriazole and their formation is considered as a detoxification process⁴. The speed and extent of aminotriazole transformation indicates that any metabolic alteration probably contributes to the phytotoxicity of the compound and any change in that rate or extent of metabolic breakdown influences phytotoxicity.

It was shown that the presence of ammonium thiocyanate enhanced the herbicidal activity of amitrole⁵ and considerably reduced the formation of aminotriazolyalanine, **Error! Bookmark not defined.**

Babiker and Duncan showed that ammonium thiocyanate enhanced the absorption of amitrole by bracken⁶ while Donnalley and Ries found it has no effect on amitrole absorption by quackgrass. Donnalley explained the enhanced herbicidal activity of amitrole by an increased translocation of amitrole when applied by foliar spraying with ammonium thiocyanate. He showed that the phytotoxicity of amitrole was influenced by the application site of amitrole and ammonium thiocyanate. The amount of chlorosis was only increased when ammonium thiocyanate was applied with amitrole to the foliage. When ammonium thiocyanate is applied one day before aminotriazole or in combination with aminotriazole, it increased translocation over aminotriazole alone or when ammonium thiocyanate is applied one day after. Donnalley and Ries suggested that ammonium thiocyanate reduced rapid damage to cells at the site of absorption thereby permitting the foliage to absorb and translocate aminotriazole over a longer period of time.

Ammonium thiocyanate was shown to inhibit the oxidation of aminotriazole into two free –radical generating systems (riboflavin photosensitized oxidation and oxidation by hydroxyl radicals). By preventing aminotriazole free-radical formation ammonium thiocyanate would prevent its subsequent conjugation with amino acids and other compounds and delay a detoxification process of amitrole thus increasing the concentration of amitrole in plants. This would explain the fact that in presence of ammonium thiocyanate the formation of aminotriazolyalanine is reduced **Error! Bookmark not defined.**

Taken collectively these findings could be considered as pointing towards the role of ammonium thiocyanate as that of preventing rapid formation of conjugates at the absorption site, probably by inhibiting free- radical reactions. This could permit a more efficient distribution of the toxic (efficient) moiety from the site of absorption to the conducting tissues and hence allow more efficient translocation of amitrole to the root system.^{7,8}

So in presence of ammonium thiocyanate, the plant metabolism of amitrole is delayed or inhibited, thus concentrating amitrole in plant. But as the residue definition is already set as amitrole only, no change in the nature of residue is expected when amitrole is applied together with ammonium thiocyanate.

RMS (FR): This statement is considered acceptable since statements from the scientific literature are in accordance with results from additional residue trials provided on grapes (B.7.6.3.1) and also on olives (B.7.6.4.2) realized with the synergist and for which amitrole, aminotriazolyalanine and triazolyalanine showed a no residue situation.

³ Carter, M.C. 1965. Studies on the metabolic activity of 3-amino-1,2,4-triazole. *Physiol. Plant.* 1965. 18, 1054.

⁴ Massink, P. *Acta Bot Nederl.* 1963. 12, 64.

⁵ Donnalley, W.F. 1934. Studies on the herbicidal enhancement of 3-amino-1,2,4-triazole by amonuium thiocyanate with *agropyron repens*. 65-1728.

⁶ Babiker, A.G.T and Duncan, H.J. 1975. Penetration of bracken fronds by amitrole as influenced by pre-spraying conditions, surfactants and other additives. *Weed research.* vol15, 123-127.

⁷ Donnalley, W.F & Ries, S.K. 1964 Jul 31. Amitrole translocation in *agropyron repens* increased by the addition of ammonium thiocyanate. *Science.* 145 (3631) : 497-8

⁸ Cook, G.T & Duncan, H.J. 1979. Mode of action of thiocyanates and iodides in amitrole formulations. *Pestic. Sci.* 281-290

B.7.1.3. Report on olive with radiolabelled amitrole

A study was submitted for ECCO Meeting (December 1999) and referenced in the relied on of the addendum of March 2000 but not peer reviewed.

Study	KIIA 6.3.3/05 [¹⁴ C]-Amitrole : Isolation and characterisation of an Unknown Major Radioactive Fraction in Olives
Reference	Schanne C. (1998)
Date performed	1994
Test facility	Springborn Laboratories (Europe) AG, Health and Environmental Sciences, Seestrasse 21, CH-9326 Horn, Switzerland – source CFPI AGRO
Report reference	Springborn Rport # 97-044-1002 # 1002.015.756
Guideline(s)	EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants
Deviations from the guideline	Yes, study not considered as a metabolism study
GLP	Yes
Test material	3-Amino-1,2,4-[3- ¹⁴ C]-Triazole, Radiopurity:98%, molecular weight 84.7 g/mol), specific activity 228 µCi/mg;
Study acceptable	No

Characteristics: the purpose of this study was to characterise a significant unknown polar radioactivity fraction as formed due to degradation and/or metabolism of [¹⁴C]-amitrole in homogenised whole olive fruits during a 3-hour incubation period at ambient temperature.

Study design: untreated olives as obtained during a field trial in 1994 from control trees were used as raw agricultural commodity to conduct the experiments. Homogenised whole olive fruits (including the stones) were spiked with [¹⁴C]-amitrole corresponding to a concentration of c.a. 5 mg [¹⁴C]-amitrole per kg whole homogenised olives.

Two different spiking procedures with duplicates were performed:

- First method: 10g olive homogenate was mixed with 4 g hydromatrix. Thereafter the mixture was put into an ASE (Accelerated Solvent Extractor) steel tube and spiked with 500µL of the radioactivity solution, divided in 10 spots by means of a syringe. The tubes were closed and the homogenate was aged for 3 hours at room temperature.
- Second method: 10g whole olive homogenate was weighted in an appropriate glass vessel and treated drop by drop with 500L of the solution by means of a syringe. The homogenate was mixed thoroughly by means of a spatula immediately after treatment and after 1, 2, and 3 hours of incubation. After 3 hours, the aged homogenate was mixed with c.a. 4g of hydromatrix and filled into the ASE tube.

The plant material was extracted after treatment by means of an ASE with a mixture of acetone/water 3/1 at 130°C and under high pressure c.a.100bar.

Due to the fact that a small volume of supernatant oil was found on top of the acetone/water extract, both extract fractions were separated and their volumes determined. The resulting extracts were quantified by LSC. The amount of radioactivity found in the oil was negligible and therefore the oil was discarded. Characterisation and identification was realized by various analytical techniques: TLC followed by normal and reverse phase and with various solvent systems, strong mineral acid hydrolysis following by TLC, HPLC and MS. Conjugates of amitrole and fatty acids, which represent major components of the olive fruits, were produced and used as analytical standards in order to identify components of the polar radioactive material by chromatography and MS techniques.

Non-extractable radioactivity was quantified by combustion of the post-extraction plant debris followed by radio assay. Mass balances were calculated based upon extractable and non-extractable radioactivity, and related to the spiked radioactivity.

Results: the metabolite profile was studied in various solvent systems of neutral, acidic and alkaline character on normal as well as on reversed phase TLC plates. Solvents containing ammonia and alkaline conditions showed the best resolution which confirms the alkaline and polar profile of the metabolites. At least, the chromatographic results show that the polar radioactive fraction as obtained after aging of [¹⁴C] amitrole in homogenised whole olives consists of numerous minor unknown extractable components (c.a. 20 minor components) of which none exceed 4.5% of the applied radioactivity. The parent amitrole was not found. Strong hydrolysis condition showed the presence of carbohydrates and fatty acids. A parallel experiment showed that conjugates were formed *in vitro* using activated forms of the fatty oleic, palmitic, stearic and linoleic acids with 24 hours at 50°C. Effectively the spectra showed that one and two molecules of amitrole were conjugated to one molecule of oleic acid but do not conjugate with triglycerides. Nevertheless and despite that oleic acid is the major fatty acid naturally occurring in the olives, its conjugate with [¹⁴C] amitrole was not detected when submitted to the same extraction conditions as in olive matrix, purified by preparative TLC and analysed by MS.

This is most likely due to the low amount of this component in the extract together with a considerable amount of biological matrix even after preparative isolation of the extract component.

Table 7.1.3-1: Material balance of radioactivity in whole homogenized olives after treatment of 5mg/kg [¹⁴C] amitrole diluted with 9 parts (AM-1 and AM-2) or 49 parts (AM-3 and AM-4) of unlabelled amitrole and incubation for 3 hours at ambient temperature

Radioactivity portion	Sample code			
	AM-1	AM-2	AM-3	AM-4
Extractable radioactivity (%)				
-Oil (%)	4.2	0.6	<0.1	<0.1
-Acetone/water 3/1 (%)	32.2	52.9	53.7	49.0
Subtotal (%)	36.4	53.5	53.7	49.0
Non-extractable radioactivity (%)	54.6	39.0	51.5	56.8
Recovery (%)	90.9	92.5	105.4	105.9

Discussion and conclusion: this report may confirm that the disappearance of the parent in homogenized whole olives is fairly complete and rapid (within 3 hours). Furthermore the parent compound seems to be degraded/metabolised into a numerous of minor unknown extractable components (c.a. 20 minor components) of which none exceeds 4.5% of the applied radioactivity.

These results could bring additional information about the amitrole behaviour in high lipid content matrix. Nevertheless this experiment was not performed with incurred radiolabelled amitrole in olives (i.e. after a field treatment). In addition the extraction was performed with an ASE system (i.e. in warm condition and very high pressure c.a. 100bar). In all likelihood, these conditions could be destructive. A recovery test with the parent amitrole extracted from olives in mild conditions would be required.

Therefore, this study is not considered as a complete and rigorous radiolabelled metabolism study in compliance with EU guideline appendix A.

RMS (FR): for information, this study was initially provided in addendum ECCO meeting not to demonstrate the metabolism of amitrole in olives but to give an appropriate analytical method to analyze residues of amitrole in olives. Consequently in ECCO review conclusions, this study was only considered to rely with an analytical question and not for metabolism purpose. Nevertheless this study was disposed without summary in relied on concerning residue section in the addendum of March 2000. For information, and concerning this analytical point, it was not possible to properly validate the proposed method due to immediate conjugation of amitrole to many natural components. Since this time, a validated method was provided for olives (see B.7.6.1.3; B.7.6.4.2 ; B.7.6.4.3)

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES IN LIVESTOCK (ANNEX IIA 6.2 AND ANNEX IIIA 8.1)

Studies on nature or magnitude of residues in commodities of animal origin are not available, nor required. Following the estimation of the dietary burden arising from the authorised uses of amitrole, MRLs for livestock products are not required because livestock is not expected to be exposed to significant levels of amitrole residues.

B.7.3. DEFINITION OF THE RESIDUE (ANNEX IIA 6.7, ANNEX IIIA 8.6)

B.7.3.1. Definition of the residue in plants and plant products

The peer review concluded for the first inclusion that the metabolism of amitrole was sufficiently elucidated in primary crops and supported the residue definition for enforcement and risk assessment as amitrole only in fruits and fruiting vegetables following application to soil.

This residue definition was limited to the evaluated uses, i.e. soil application to fruiting crops, since the metabolism has only been investigated in apples. Olive is a new intended use since the first authorisation. Olive can be considered as a significantly different medium and a specific metabolism study might be required.

Nevertheless,

- Regarding the metabolism available in apple and the similar mode of application to the soil for olives, direct interception or systemic concentration in olives would be of low importance since, with a log P_{ow} = - 0.969 (pH 7 at 23°C), amitrole is not considered as a lipophilic substance and in all likelihood it would not more concentrate in high lipid content matrices like olives than in high water content matrices like apples.
- Residues studies provided on olives since the first inclusion showed that residue levels of amitrole, aminotriazolanine and triazolanine remain below the LOQ of 0.01mg/kg after application at a_n exaggerated rate (c.a.1.5N).

Consequently further specific metabolism studies in olives are not considered essential at this time and the need to include additional metabolites in a separate residue definition (e.g. triazolylalanine) might be considered when specific data addressing the toxicology of the triazole derivative metabolites (TDMs) will be available.

Taking into account the available metabolism study on apple and also the no residue situation via new residue data provided in the context of this addendum and concerning amitrole, aminotriazolalanine and triazolalanine, it can be concluded that the metabolic pathway in crops under consideration (fruits and fruiting vegetables including olives) is sufficiently addressed.

The residue definition derived is also in line with those derived by the JMPR (FAO, 1998) and EFSA reasoned opinion (EFSA 2012).

Table 7.3.1-1: Residue definition for monitoring and risk assessment proposed in plant for amitrole

	Definition	Conversion factor for enforcement to risk assessment	Source
Plant residue definition for monitoring	amitrole	-	EU regulation n° 149/2008
Plant residue definition for risk assessment	amitrole	-	FR (1996) EFSA (2012)

B.7.3.2. Definition of the residue in animal products

Studies on the nature or magnitude of residues in commodities of animal origin are not available, nor required. Following the estimation of the dietary burden arising from the authorised uses of amitrole, residue definitions for livestock products are not required because livestock is not expected to be exposed to significant levels of amitrole residues.

Table 7.3.2-1 Residue definition for monitoring and risk assessment proposed in animal for amitrole

	Definition	Conversion factor for enforcement to risk assessment	Source
Animal residue definition for monitoring	Not necessary	-	EU regulation n° 149/2008
Animal residue definition for risk assessment	Not necessary	-	FR (1996) EFSA (2012)

B.7.4. USE PATTERN

Table 7.4-1 : Summary of intended uses amitrole

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/L (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./hl min max	Water l/ha min max	Kg a.i./ha min max		
Orchards (citrus fruits, pome and stone fruits, assorted fruits-edible or inedible peel, tree nuts)	All MS	CA 2735	F	Non selective control of annual and perennial monocotyledones and dicotyledones weeds (growth stage 40-60)	Soluble concentrate (SL)	229 g:L (HPLC)	broadcast spraying under the row or on localized areas with tractor mounted boom	94-75	1 (or several splitted applications)	2-3 months	0.24-1	300 to 500	1.2-3	35	CA2735 is applied either once per year at 2977 g a.i./ha (i.e. 13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 g a.i./ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/L (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./ha min max	Water l/ha min max	Kg a.i./ha min max		
Grapes (table and vine)	All MS	CA 2735	F	Non selective control of annual and perennial monocotyledones and dicotyledones weeds (growth stage 40-60)	Soluble concentrate (SL)	229 g/L (HPLC)	broadcast spraying under the row or on localized areas with tractor mounted boom	92-79	1 (or several splitted applications)	2-3 months	0.24-1	300 to 500	1.2-3	42	CA2735 is applied either once per year at 2977 g a.i./ha (i.e. 13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 g a.i./ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/L (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./ha min max	Water l/ha min max	Kg a.i./ha min max		
Olives (table and for oil production)	South EU MS	CA 2735	F	Non selective control of annual and perennial monocotyledones and dicotyledones weeds (growth stage 40-60)	Soluble concentrate (SL)	229 g:L (HPLC)	broadcast spraying under the row or on localized areas with tractor mounted boom	Spring and autumn	1-2 (or several splitted applications)	2-3 months	0.18-0.5	300 to 500	0.9-1.375	35	CA2735 is applied either once or twice per year at 1375 g a.i./ha (i.e. 6 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 2750 g a.i./ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/L (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.s./ha min max	Water l/ha min max	Kg a.i./ha min max		
Non crop uses (impermeable and permeable surfaces: road, railways)	All MS	CA 2735	F	Non selective control of annual and perennial monocotyledones and dicotyledones weeds (growth stage 40-60)	Soluble concentrate (SL)	229 g:L (HPLC)	broadcast spraying under the row or on localized areas with tractor mounted boom	Not applicable	1 (or several splitted applications)	2-3 months	0.36-1	300 to 500	1.8-3	-	CA2735 is applied either once per year at 2977 g a.i./ha (i.e.13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 g a.i./ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.

* For uses where the column „Remarks“ in marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

- (a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)
- (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
- (c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
- (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
- (e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
- (f) All abbreviations used must be explained
- (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
- (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

- (i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants
- (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (k) Indicate the minimum and maximum number of application possible under practical conditions of use
- (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha)
- (m) PHI - minimum pre-harvest interval

B.7.5. IDENTIFICATION OF CRITICAL GAPS

CROP	GAP
Orchards (citrus fruits, pome and stone fruits, assorted fruits-edible or inedible peel, tree nuts)	1.2-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year with BBCH growth stage between 94-75 and 35 day PHI
Grapes (table and vine)	1.2-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year with BBCH growth stage between 92-79 and 42 day PHI
Olives (table and oil production)	0.9-1.375 kg a.i./ha, 1-2 application or maximum application rate /year of 2750 g a.i./ha/year in spring and autumn and 35 day PHI
Non crop uses (impermeable and permeable surfaces: road, railways)	1.8-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year no edible commodity : no PHI

B.7.6. RESIDUES RESULTING FROM SUPERVISED TRIAL (ANNEX IIA 6.3, ANNEX IIIA 8.2)**B.7.6.1. Sample storage stability for analysis (annex IIA 6.0)**

Since the first inclusion of amitrole, stability studies were provided in the context of the first national (France) authorization of the product WEEDAZOL TL on orchards and vineyard.

B.7.6.1.1. Stability in apple – Bieber (2000)

Study	KIIA 6.1.1/02 Amitrole – Stability in Apples Stored Under Frozen Conditions
Reference	Bieber, R.C (2000)
Date performed	1994
Test facility	Springborn Laboratories, Inc. Wareham, Massachusetts.
Report reference	12983.0896.6128.395 95-08-6065,
Guideline(s)	EEC 7032/VI/95 rev. 5 Appendix H (EU): Storage stability of residue samples
Deviations from the guideline	Significant blank contamination with the first experiment spiked with 0.05mg/kg, only results from the experiment spiked with 1 mg/kg were considered.
GLP	Yes
Test material	Amitrole, Lot/Batch No. RD-467-067-1, CAS No61-82-5, a white powder reported by the study sponsor to have a purity of 99.6% w/w
Study acceptable	Yes

Materials and method: apples were spiked with amitrole (0.05 and 1 mg a.s./kg) and stored in frozen condition. Extraction and analysis was realized after 0, 3, 6, 12 and 16 months of storage duration. The extraction was realized with denatured ethanol and centrifuged. The extract was evaporated to dryness and reconstituted in reagent grade water. When necessary, a cation exchange clean up procedure was realized. Amitrole was analysed with a validated method using reverse phase and isocratic HPLC coupled with electrochemical detection. The limit of quantification (LOQ) for amitrole in apples is 0.01 mg/kg.

Results: results are presented in the following table

Table 7.6.1.1-1 : Residues of amitrole in apple after storage in frozen condition (Bieber, stability study on apple)

Storage period (months)	Residue Level of amitrole in Freezer Storage Stability Sample (mg/kg) (range plus median)		Residue Level in Freezer Storage Stability Sample (% of nominal spiking level)			Procedural Recovery for freshly spiked control sample (mg/kg)	Procedural Recovery for freshly spiked control sample (%)
0	0.739; 0.828; 0.843	0.828	100%				
3	0.796; 0.786	0.791	96%	95%	96%	0.744	90%
6	0.785; 0.819	0.802	95%	99%	97%	0.818	99%
12	0.663; 0.635	0.649	80%	77%	78%	0.763	92%
16	0.679; 0.758	0.7185	82%	92%	87%	0.725	88%

Conclusion: Apples were spiked with amitrole to assess the stability of amitrole residues during prolonged storage in frozen condition. Results do not reveal significant reduction in amitrole levels over the sampling period. Amitrole is considered stable in apple (a representative crop with high water content) over a period of 16 month of storage in frozen condition.

B.7.6.1.2. Stability in grape – Clemson (1997)

Study	KIIA 6.1.1/01 Amitrole: determination of freezer storage stability of amitrole residues in grapes
Reference	Clemson, A.D. (1997)
Date performed	1994
Test facility	Huntington LifeSciences
Report reference	No: 95/CPF018/1407
Guideline(s)	EEC 7032/VI/95 rev. 5 Appendix H (EU): Storage stability of residue samples
Deviations from the guideline	No
GLP	Yes
Test material	Amitrole, Lot/Batch No. RD-467-067-1, Purity (HPLC) 99.55% (Huntingdon Life Sciences reference No 007128)
Study acceptable	Yes

Materials and methods: samples collected during field trial No.CPF/OI7 were used in this stability study. These samples were received frozen in good condition on 3 February 1993 and 28 April 1994. The grapes were stored at about - 20°C until required. Since residue level wasn't sufficient on the previously treated grapes, on 17 November 1993, whole, frozen grapes (3 kg) from Trial SE-0035, 30750 Gajan, France, (received 3 February 1993) were dipped in a dilute aqueous solution of WEEDAZOL TL (a 240 g/L commercial product), containing 3.2 mg/L amitrole. The procedure for the determination of amitrole in grapes was based upon a validated HPLC with electrochemical detection method for the determination of amitrole residues in vegetables, soil and water. The limit of quantification (LOQ) for amitrole in apples is 0.01 mg/kg.

Results: results are presented in the following table

Table 7.6.1.2-1 : Residues of amitrole in grape after storage in frozen condition (Clemson, stability study on grape)

Storage period (months)	Residue Level in Freezer Storage Stability Sample (mg/kg) (range plus median)		Residue Level in Freezer Storage Stability Sample (% of nominal spiking level)			Procedural Recovery for freshly spiked control sample(mg/kg)	Procedural Recovery for freshly spiked control sample (%)
0	0.184; 0.261, 0.262, 0.329	0.2620	100%				
1	0.285, 0.392	0.3390	109%	150%	129%	0.164	63%
4	0.329, 0.354	0.3420	126%	135%	130%	0.138	53%
8	0.170, 0.234	0.2020	65%	89%	77%	0.172	66%
16	0.157, 0.176	0.1660	63%	62%	65%	0.192	73%
24	0.147, 0.210	0.1790	56%	80%	68%	0.165	63%

Conclusion: grapes were spiked with amitrole to assess the stability of amitrole residues during prolonged storage at about -20°C. Analyses were conducted at intervals over two years and the results revealed a reduction in amitrole levels over the sampling period. The rate of decline was confused with an apparent slight increase at the early time points and a trend for low fresh recoveries. At the end of the study the amitrole concentration had fallen to about 60% of the initial. Amitrole is considered stable in grape (a representative crop with acid content) over a period of 8 months in frozen condition.

B.7.6.1.3. Stability in grape and olive – Amic (2011)

Study	KIIA 6.1.1/03 Determination of Storage Stability of Amitrole in Grapes and Olives under Freezer Storage Conditions
Reference	Amic, S. (2011)
Date performed	2011
Test facility	EUROFINS ADME BIOANALYSIS
Report reference	No: S10-03264
Guideline(s)	EEC 7032/VI/95 rev. 5 Appendix H (EU): Storage stability of residue samples
Deviations from the guideline	No
GLP	Yes
Test material	Amitrole, Lot/Batch No. RD-467-067-1, CAS No61-82-5, a white powder reported by the study sponsor to have a purity of 99.6% w/w
Study acceptable	Yes

Materials and method: samples of grapes and olives were individually fortified with a known amount of amitrole and its metabolites triazolylalanine and aminotriazolylalanine. Homogenized grape and olive samples were weighed into plastic centrifuge bottles. The appropriate amount of reference item solution was added to fortify this sample at 0.1 mg/kg. Samples were securely capped and placed at -20 ± 5°C until analysis. For each compound, three replicate samples of fortified matrix were analysed at zero time and two replicate samples were removed from storage at a nominal temperature of -20 ± 5°C after 6.5 months storage and analysed along with freshly prepared samples fortified at 0.1 mg/kg for grape and olive matrices. The recovery obtained with these fortified samples was compared with the recovery obtained with freshly fortified samples. The validated analytical method AGR/MOA/AMT-8 for grape matrix and AGR/MOA/AMT-2 for olive matrix involved an extraction by homogenization with acetone/ultra pure water (75/25, v/v) mixture. After filtration an aliquot was

clean-up by liquid-liquid partition with dichloromethane and then with hexane. The aqueous phase was acidified and purified over an MCX cartridge (500 mg / 6 mL). After evaporation, the dry extract was dissolved in ultra pure water and then analysed by LC-LC-MS/MS (for amitrole) and LC-MS/MS (for the metabolites) with electropositive and electronegative ion spray. The limit of quantification was 0.01 mg/kg for amitrole and also for its metabolites triazolyalanine and aminotriazolyalanine in grape and olive matrices.

Results: results are presented in the following tables

Table 7.6.1.3-1: Residues of amitrole in grape after storage in frozen condition (Amic, stability study on grape)

Compound	Months	Uncorrected compound concentration (mg/kg)					Procedural recoveries (%)		
		rep1	rep2	rep3	median	(%)	rec1	rec2	Mean
Amitrole	0	0.10	0.10	0.10	0.10	100	98	103	101
	6.5	0.11	0.11		0.11	113	106	108	107
Triazolyalanine	0	0.08	0.07	0.07	0.08	100	84	84	84
	6.5	0.09	0.08		0.08	111	82	85	84
Aminotriazolyalanine	0	0.11	0.10	0.10	0.10	100	104	102	103
	6.5	0.09	0.10		0.10	93	85	90	88

Table 7.6.1.3-2: Residues of amitrole in olive after storage in frozen condition (Amic, stability study on olives)

Compound	Months	Uncorrected compound concentration (mg/kg)					Procedural recoveries (%)		
		rep1	rep2	rep3	median	(%)	rec1	rec2	Mean
Amitrole	0	0.10	0.10	0.10	0.10	100	99	101	100
	6.5	0.10	0.09		0.09	96	98	98	98
Triazolyalanine	0	0.09	0.10	0.09	0.09	100	90	95	93
	6.5	0.09	0.09		0.09	93	85	85	85
Aminotriazolyalanine	0	0.10	0.10	0.10	0.10	100	101	101	101
	6.5	0.10	0.10		0.10	99	98	98	98

Conclusion: amitrole and its metabolites triazolyalanine and aminotriazolyalanine are considered stable in grape (a representative crop with acid content) and olives (a representative crop with high lipid content) over a period of 6.5 months in frozen condition.

B.7.6.1.4. Overview of acceptable maximum storage duration

Amitrole		
Crop	Characteristics of the crop group	Acceptable Maximum Storage duration
Apple	Crop with high water content	16 months for amitrole
Grape	Crop with acid content	8 months for amitrole 6.5 months for triazolyalanine 6.5 months for aminotriazolyalanine
Olive	Crop with high lipid content	6.5 months for amitrole 6.5 months for triazolyalanine 6.5 months for aminotriazolyalanine
Animal Products		
Not required		

B.7.6.2. Supervised trials on orchards**Table 7.6.2.-1:** Overview of the uses of amitrole on orchards in EU

Crop	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
Pome fruits	EU N (FR)	1-2	1200-4000 g a.s/ha Soil treatment spraying	60 days	Band application (on row) or spot treatment – application from autumn to spring	35
	EU S (FR/ES)		1200-3600 g a.s/ha Soil treatment spraying		BBCH 94 to 75 (all year around except during fruit ripening) - Max 3600 gas/ha/year	
	EU (DAR)	2	3420 g a.s/ha Soil treatment spraying		From autumn to spring (all year around except during fruit ripening)	
	Intended FR (Troll in progress)					
	EU AIR II	1-2	1200-3000 g a.s/ha Soil treatment spraying	2-3 months	BBCH 94-75 – Broadcast spraying under the row or on localised areas with tractor mounted boom. CA2735 is applied either once per year at 2977 g a.i/ha (i.e. 13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 g a.i/ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	

Crop	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
Nuts	EU S (FR)	1-2	1200-4000 g a.s/ha Soil treatment spraying	60 days	Band application (on row) or spot treatment – application from autumn to spring	35
	EU (DAR)		1200-3600 g a.s/ha Soil treatment spraying		BBCH 94 to 75 (all year around except during fruit ripening) Max 3600 gas/ha/year	
	Intended FR (troll in progress)	2	3420 g a.s/ha Soil treatment spraying		From autumn to spring (all year around except during fruit ripening)	
	EU AIR II	1-2	1200-3000 g a.s/ha Soil treatment spraying	2-3 months	BBCH 94-75 – Broadcast spraying under the row or on localised areas with tractor mounted boom. CA2735 is applied either once per year at 2977 g a.i/ha (i.e. 13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 g a.i/ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	

Crop	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
Stone fruits	EU N/S (FR)	1-2	1200-4000 g a.s/ha Soil treatment spraying	60 days	Band application (on row) or spot treatment – application from autumn to spring	35
	EU (DAR)		1200-3600 g a.s/ha Soil treatment spraying		BBCH 94 to 75 (all year around except during fruit ripening) Max 3600 gas/ha/year	
	Intended FR (troll in progress)	2	3420 g a.s/ha Soil treatment spraying		From autumn to spring (all year around except during fruit ripening)	
	EU AIR II	1-2	1200-3000 g a.s/ha Soil treatment spraying	2-3 months	BBCH 94-75 – Broadcast spraying under the row or on localised areas with tractor mounted boom. CA2735 is applied either once per year at 2977 g a.i /ha (i.e. 13 L product/ha) or several times at lower rates but respecting the maximum application rate	

					/year of 3000 g a.i./ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	
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Crop	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
citrus	EU S (ES)	1-3	1200-3600 g a.s/ha Soil treatment spraying	60 days	Application : all year around except during fruit ripening – broadcast spraying between crop plants with tractor mounted boom – preparation include ammonium thiocyanate at 215 g/L	35
	EU (DAR)		1200-3600 g a.s/ha Soil treatment spraying		BBCH 94 to 75 (all year around except during fruit ripening) Max 3600 gas/ha/year	
	EU AIR II (all MS)	1-2	1200-3000 g a.s/ha Soil treatment spraying	2-3 months	BBCH 94-75 Broadcast spraying under the row or on localised areas with tractor mounted boom. CA2735 is applied either once per year at 2977 gai/ha (ie 13 L product/ha) or several times at lower rates but respecting the maximum application rate /year of 3000 gai/ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	

B.7.6.2.1. Studies previously submitted in the monograph on pome fruits and stone fruits

Study	AnnexIIA, 6.6.1.1 - Residue trials with Ustinex K.R. in apples in Germany
Reference	Anonymous (1979, 1982, 1990)
Report reference	No: 0625-79, 0626-79, 0627-79, 0637-79, 0600-82, 0726-90, 0727-90
GLP	No
Study	AnnexIIA, 6.6.1.2 - Residue trials with Ustinex K.R. in pear conducted in Germany
Reference	Anonymous (1982)
Report reference	No: 0601-82
GLP	No
Study	AnnexIIA, 6.6.1.2 - Residue trials with Aminotriazole in apples in Germany
Reference	Anonymous (1983)
Report reference	No: 0600-83, 0601-83, 0602-83
GLP	No
Study	AnnexIIA, 6.6.1.2 - Residue trials with Aminotriazole in pear in Germany
Reference	Anonymous (1983)
Report reference	No: 0603-83, 0604-83, 0605-83
GLP	No
Study	AnnexIIA, 6.6.1.1.- Determination of residues of Aminotriazole 50 WP in/on apples under actual use conditions in Germany
Reference	Placke, F. (1993)
Report reference	No: RA-2003/91 (including studies 105147, 105155, 105163 and trials : 0514-91, 0515-91, 0516-91 and trials 0514)
GLP	yes
Study	AnnexIIA, 6.6.1.1.- Determination of residues of Ustinex KR 70 WP in/on apple and pear under actual use conditions in Portugal
Reference	Placke, F. (1994)
Report reference	No: RA-2004/92 (including studies 202290, 202304, 202312 and trials 0229-92, 0230-92, 0231-92)
GLP	yes
Study	AnnexIIA, 6.6.2.- Determination of residues of Ustinex KR 70 WP in/on peaches under actual use conditions in Spain
Reference	Placke, F. (1994)
Report reference	No: RA-2005/92 (including studies :202258, 202266, 202274, 202282 and trials 0225-92, 0226-92, 0227-92, 0228-92)
GLP	yes

B.7.6.2.2. Additional residue study on apple – Bieber (1996)

Study	KIIA 6.3.2/01 Amitrole: the accumulation and degradation of amitrole in apples.
Reference	Bieber, R.C (1996)
Date performed	1993
Test facility	Springborn Laboratories Inc.
Report reference	No: 11205 ADR – CFP404
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	No
GLP	Yes
Test material	Commercial product CA 0123 (WEEDAZOL TL) containing 240 g/l of amitrole
Study acceptable	Yes

Material and Method : the orchard plots were treated with CA-0123 (240 g/L amitrole, 215g/L of the synergist ammonium thiocyanate) at one of two rates, 2,880 g a.s./ha and 4,800 g a.s./ha or remained untreated controls. There were two, three, or four applications during 1993, beginning in early March, with the final applications being made in mid July. Apple residue samples were collected in May, June, July, August, and September, 1993. The shortest time interval between an application and a sampling was 31 days and the longest was 172 days. Days between the last application and harvest were 49, 56 and 61 days.

Table 7.6.2.2.-1: Summary of global information (Bieber study, residue trial on apple)

Comparative trials (between formulations, with and w/o adjuvant/safener/synergist)	No but the product also contains the synergist ammonium thiocyanate 215g/L
Number of applications	Product applied 2, 3 or 4 times
Dose (g as/ha)	Product CA 0123 (WEEDAZOL TL) containing 240 g/l of active substance applied at 2 rates so as to apply 2880-4800 g/ha of active substance. The product also contains the synergist ammonium thiocyanate 215g/L
Mode of application	Soil application by spraying
PHI (days) and/or growth stage (BBCH)	PHI 49, 56, 61 days
Analytical method (Code +Type)	Springborn laboratory protocol 050994/OECD (HPLC/MS)
LoQ (mg/kg)	0,01

Table 7.6.2.2.-2 : Details about the trials (Bieber study, residue trial on apple)

N° Trial	930405WE78	930405SE96	930405SW77
North/South/Indoor	N	S	S
Decline (D)/Harvest (H) trial?	D	D	D
Formulation	SL	SL	SL
Equivalence between formulations	Y	Y	Y
Accordance with intended GAP	Y	Y	Y
Correct sampling	Y	Y	Y
Samples frozen within 24h	Y	Y	Y
Storage period (in days)	Sample	Maximum 376-483 days	
	Extract	1	
Storage T° <-18°C	N	N	N
Validated analytical method	Y	Y	Y
Negative controls	Y	Y	Y
Considered trial	Y	Y	Y
Remarks	-	-	-

Results: all residues were below the LOQ of 0.01mg/kg in apples.

Table 7.6.2.2.-3 Residues data summary from supervised trials (summary) (Biever study on apple)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 240 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : CA0123 (WEEDAZOL TL)

Active ingredient : aminotriazole 240g/L

Crop / crop group : apples

Indoors / outdoors : outdoors

Other a.s. in formulation : synergist

(common name and content) : ammonium thiocyanate 215g/L

Residue calculated as : aminotriazole

1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	1. Sowing or Planting date 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
12983-0594- 6107-395 930405WE78 49250 Saint Mathurin,FR North	Apples Golden Smothee	1) 1983	Soil spraying	0,578	528	3,05	01.04.93	53	Apples	<0.01	49	LOQ = 0,01 mg/kg
		2) nr		0,576	500	2,88	01.06.93	72				
		3) 19.09.93		0,575	456	2,62	11.07.93	75				
		1) 1983	Soil spraying	0,961	544	5,23	01.04.93	53	Apples	<0.01	49	
		2) nr		0,962	444	4,27	01.06.93	72				
		3) 19.09.93		0,958	456	4,37	11.07.93	75				
12983-0594- 6107-395 930405SE96 84000 Avignon, FR South	Apples Golden Smothee	1) Winter 1986	Soil spraying	0.576	524	3.02	17.05.93	72	Apples	<0.01	61	
		2) nr		0.575	495	2.85	14.07.93	77				
		3) 13.09.93										
		1) Winter 1986	Soil spraying	0.963	525	5.06	17.05.93	72	Apples	<0.01	61	
		2) nr		0.960	529	5.08	14.07.93	77				
		3) 13.09.93										
12983-0594- 6107-395 930405SW77 82600 Aucamville,FR South	Apples Golden Smothee	1) Winter 1986	Soil spraying	0,575	520	2,99	25.03.93	53	Apples	<0.01	56	
		2) nr		0,577	513	2,96	25.04.93	65				
		3) 13.09.93		0,575	520	2,99	26.05.93	72				
				0,575	487	2,80	19.07.93	75				
		1) Winter 1986	Soil spraying	0.959	482	4.62	26.03.93	53	Apples	<0.01	56	
		2) nr		0.958	471	4.57	26.04.93	65				
		3) 13.09.93		0.960	523	5.02	27.05.93	72				
				0.965	517	4.99	20.07.93	75				

Remarks:

(a) According to EEC and Codex class classifications (both) should be used

(b) Only if relevant

(c) High or low volume spraying, spreading, dusting etc., overall broadcast, type of equipment used must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

(f) Minimum number of days after application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

B.7.6.2.3. Additional residue study on apple – Amic (2013)

Study	KIIA 6.3.2/02 Amitrole: residue study on apples in France in 2012.
Reference	Amic. S., (2013)
Date performed	2012
Test facility	Eurofins ADME BIOANALYSE
Report reference	No: S12-00485
Guideline(s)	EEC 7032/VI/95 rev. 5 Appendix H (EU): Storage stability of residue samples (draft of 22 July 1997)
Deviations from the guideline	No
GLP	Yes
Test material	Amitrole, 3-amino-1,2,4-triazole, CAS 61-82-5, commercial product CA2735 with 2299 g/L of amitrole
Study acceptable	No, not a finalized report

Table 7.6.2.3-4 : Summary of global information (Amic study on apple)

Comparative trials (between formulations, with and w/o adjuvant/safener/synergist)	-
Number of applications	1
Dose (g as/ha)	2977 g ai/ha
Mode of application	Spraying at the base of the tree
PHI (days) and/or growth stage (BBCH)	35
Analytical method (Code +Type)	Method : GR/MOA/AMT-9 Analysis of :Amitrole, Triazolylalanine and Aminotriazolylalanine
LoQ (mg/kg)	0,01

This study was provided lately in the assessment procedure and is not yet a finalized report. Consequently this report do not fulfill requirements for the moment to be validated and presented in this addendum.

B.7.6.3. Supervised trials on grapes**Table 7.6.3.-5 : Overview of the uses of amitrole on orchards in EU**

	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
Grapes	EU N/S (FR)	1-3	1200-4000 g a.s/ha Soil treatment spraying	60 days	Band application (on row) or spot treatment – application from autumn to spring	28
	EU (DAR)		1200-3600 g a.s/ha		BBCH 94 to 75 (all year around except during fruit ripening) – Established and nursery trees Low rates on annual weeds and high rates on perennial weeds - Max 3600 gas/ha/year	
	EU AIR II (all MS)	1-2	1200-3000 g a.s/ha	2-3 months	BBCH 92-79 – Broadcast spraying under the row or on localised areas with tractor mounted boom. CA2735 is applied either once per year at 2977 gai/ha (or several times at lower rates but respecting the maximum application rate /year of 3000 gai/ha/year. A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	42

B.7.6.3.1. Studies previously submitted in the monograph on grapes

Study	AnnexIIA, 6.6.3- Residue trials with Ustinex K.R. in grapes in Germany
Reference	Anonymous (1979, 1983, 1985, 1990)
Report reference	No: 0634-79, 0635-79, 0636-79, 0606-83, 0607-83, 0600-85, 0601-85, 0602-85, 0603-85, 0604-85, 0605-85, 0605, 0724-90, 0725-90
GLP	No
Study	AnnexIIA, 6.6.3.- Determination of residues of Ustinex KR 80 WP in grapes under actual use conditions in Germany
Reference	Placke, F.(1993)
Report reference	No: RA-2127 (including studies 102520, 102539, 102547 and trials : 0252-91, 0253-91, 0254-91)
GLP	Yes
Study	AnnexIIA, 6.6.3.- Determination of Aminotriazole residues in grapes
Reference	Toussaint.G.(1992)
Report reference	No: 911101 – 80/900

GLP	Yes
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B.7.6.3.1. Additional residue study on grape – Provot (1997)

Study	KIIA 6.3.1/03 - Determination de residus d'aminotriazole et de terbutylazine dans les echantillons de raisin traités avec le produit expérimental CA1397.
Reference	Provot (1997)
Date performed	1993
Test facility	Centre International de Toxicologie (C.I.T)
Report reference	No: 95-08-6065/12983-0594-6107-395
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	No
GLP	Yes
Test material	Commercial product CA 1397 SL= CARAZOL PRO with 200g/L aminotriazole
Study acceptable	Yes

Material and Method: 5 trials with grapes were treated with a soil application of CA-1397 (SL commercial product CARAZOL PRO 200 g/L of amitrole and 100g/L of terbutylazine) at a rate of 3000 g of amitrole/ha. Applications were realized at very early stage between dormancy (=BBCH 00) and wool stage (BBCH 05). Grapes were harvested at mature stage, stored in frozen condition, extracted with dichloromethane and analysed by LC-MS/MS. The LOQ of the method was 0.025mg/kg.

Table 7.6.3.1-6 : Summary of global information (Provot study, residue trial on grape)

Comparative trials (between formulations, with and w/o adjuvant/safener/synergist)	No but the product also contains 100g/L of terbutylazine
Number of applications	1
Dose (g as/ha)	3000 : 15L/ha of product CA 1397 SL=CARAZOL PRO (200g/L aminotriazole and 100g/L terbutylazine)
Mode of application	Soil application by spraying
PHI (days) and/or growth stage (BBCH)	Application at 177-193 days before harvest
Analytical method (Code +Type)	Method : H600 (LC-MS/MS) for amitrole
LoQ (mg/kg)	0,01 for each compound

Table 7.6.3.1-7 : Details about the trials (Provot study, residue trial on grape)

N° Trial	SE82	SE83	SW84	SW85	WE86
North/South/Indoor	S	S	S	S	N
Decline (D)/Harvest (H) trial?	H	H	H	H	H
Formulation	SL	SL	SL	SL	SL
Equiv.between formulations	Y	Y	Y	Y	Y
Accordance with intended GAP					
Correct sampling	Y	Y	Y	Y	Y
Samples frozen within 24h	Y	Y	Y	Y	Y
Storage period (in days)	Sample	199-222 : terbutylazine 242-787 : aminotriazole			
	Extract	<2			
Storage T° <-18°C	Y	Y	Y	Y	Y
Validated analytical method	Y	Y	Y	Y	Y
Negative controls	Y	Y	Y	Y	Y
Considered trial	Y	Y	Y	Y	Y
Remarks	-	-	-	-	-

Results: all residues were below the LOQ of 0.025 mg/kg in grapes.

Table 7.6.3.1.-8 : Residues data summary from supervised trials (summary) (Provot study on grapes)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 200 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : CA1397 (CARAZOL PRO)

Active ingredient : aminotriazole 200g/L

Crop / crop group : grappes

Indoors / outdoors : outdoors

Other a. s. in
formulation(common name and : terbutylazine 100g/L
content)

Residue calculated as : aminotriazole

1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment kg a.s./hl Water (l/ha) kg a.s./ha			Dates of treatment(s) or no of treatment(s) (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (1)Ref method (2) LoQ (g)
11205/ADR/SE82 26260 Curson/FR south	Grape Syrah	1) – 2) – 3) 21/09/93	spraying	0.6	500	3.0	22/03/93	BBCH01 (beginning of bud swelling)	bunch of grapes	< 0.025	183	(1) H 600 (2)0.025 mg/kg
11205/ADR/SE83 30730 Gajan/ FR south	Grape Cinsaut	1) – 2) – 3) 16/09/93	spraying	0.6	500	3.0	23/03/93	BBCH00 (dormancy)	bunch of grapes	< 0.025	177	(1) H 600 (2)0.025 mg/kg
11205 ADR/SW84 31620 Fronton/FR south	Grape Négrette	1) – 2) – 3) 20/09/93	spraying	0.6	500	3.0	26/03/93	BBCH00 (dormancy)	bunch of grapes	< 0.025	178	(1) H 600 (2)0.025 mg/kg
11205/ADR/SW85 82370 Orgueil/ FR south	Grape Cabernet Franc	1) – 2) – 3) 05/10/93	spraying	0.6	500	3.0	26/03/93	BBCH00 (dormancy)	bunch of grapes	< 0.025	193	(1) H 600 (2)0.025 mg/kg
11205/ADR/WE86 49320 St Saturnin/Loire FR north	Grape Cabernet Franc	1) – 2) – 3)11/10/93	spraying	0.6	500	3.0	07/04/93	BBCH05 (wool stage)	bunch of grapes	< 0.025	187	(1) H 600 (2)0.025mg/kg

Remarks:

(a) According to EEC and Codex class classifications (both) should be used

(b) Only if relevant

(c) High or low volume spraying, spreading, dusting etc., overall broadcast, type of
equipment used must be indicated

(d) Year must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

(f) Minimum number of days after application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning
the metabolites included, the method of storage, storage stability, analysis date

B.7.6.3.2. Additional residue study on grape – Wilson (1999)

Study	KIIA 6.3.1/01 - Amitrole – residue exploratory trial on processing olives in Spain in 2010.
Reference	Wilson A.J. (1999)
Date performed	1999
Test facility	Huntingdon Life Sciences, Ltd
Report reference	No: CPF/024
Guideline(s)	EEC 7035/VI/95 rev. 5 Appendix E (EU): Processing studies
Deviations from the guideline	No
GLP	Yes
Test material	Commercial product WEEDAZOL TL with 240g/L amitrole
Study acceptable	No

Material and Method: in 1997, 8 trials were performed in France (6 trials) and Spain (2 trials) to establish the magnitude of the residue data for amitrole in grapes. Additionally, processing steps were conducted on four of the trials in France to establish magnitude of the residue of aminotriazole in grape juice, must and wine. Commercial product WEEDAZOL TL was applied (15L/ha, eq. to 3.54-3.76 kg a.i./ha or 30L/ha eq. to 7.26-7.35 kg a.i./ha) with a backpack boom sprayer to several test plots at each trial location in a single application. They were treated at 3 different dates with the last one about 30 days before harvest. Deposition cards for control were also placed in each plot during the application and subsequently analysed to confirm the effectiveness of the application.

Table 7.6.3.2-9 : Summary of global information (Wilson study, residue trial on grape)

Comparative trials (between formulations, with and adjuvant/safener/synergist)	No
Number of applications	1
Dose (g as/ha)	3530-3760 and 7160-7350 g/ha (15 L/ha or 30 l/ha of the commercial product WEEDAZOL TL containing 240g/l amitrole and 215 g/L of the synergist ammonium thiocyanate)
Mode of application	spraying with a backpack boom sprayer
PHI (days) and/or growth stage (BBCH)	30
Analytical method (Code +Type)	Huntingdon Life Sciences analytical method (described in report 97/CPF017/0935) (GC/MS)
LoQ (mg/kg)	LOQ of 0.01 mg/kg for grapes, wine, juice and must, and an LOD of 0,005 for grapes and of 0,00125 for juice, wine and must
Remark	previous treatments with products containing amitrole before

Harvested grapes were stored in frozen conditions. Approximately 70kg of grapes were taken from each relevant plot for the four processing trials. 10kg of grapes were taken from each processing sample for juice processing. The remainder was processed into must and red and white wine. Analysis was realized with validated GC/MS method with a LOQ of 0.01mg/kg for juice, must and wine.

Juice: grapes were stemmed, crushed, weighed and pectolytic enzymes were added. Depectinisation was realized between 55°C and 60°C for 2 hours in a sterilizer. The content was then pressed and recovered juice was sterilized during 5 min between 85-88°C.

Wine process: grapes were pressed and placed into a stainless steel or glass tank. With regards to oxidation, the must was sulphited by addition of potassium metabisulphite. Pectolytic enzymes were added and the must decanted for 12-24 hours. After decantation and transfer, dry yeasts (*Saccharomyces cerevisiae*) were added to the clear juice. When the alcoholic fermentation was finished, potassium metabisulphite was added to block the malolactic fermentation and obtain the final wine after decantation and stabilization steps.

Table 7.6.3.2-10: Details about the trials (Wilson study, residue trial on grape)

N° Trial	CPF/024-01	CPF/024-02	CPF/024-03	CPF/024-04	CPF/024-05	CPF/024-06	CPF/024-07	CPF/024-08
North/South/Indoor	N (FR)-1997	N (FR)-1997	S (FR)-1997	S (FR)-1997	S (FR)-1997	N (FR)-1997	S (FR)-1997	S (FR)-1997
Decline (D)/Harvest (H) trial?	H + red wine processing (gamay)	H + white wine processing (chardonnay)	H + red wine processing (merlot)	H + red wine processing (merlot)	H	H	H	H
Formulation	SL	SL	SL	SL	SL	SL	SL	SL
Equivalence between formulations	Y	Y	Y	Y	Y	Y	Y	Y
Accordance with intended GAP	Y	Y	Y	Y	Y	Y	Y	Y
Correct sampling	Y	Y	Y	Y	Y	Y	Y	Y

N° Trial		CPF/024-01	CPF/024-02	CPF/024-03	CPF/024-04	CPF/024-05	CPF/024-06	CPF/024-07	CPF/024-08
Samples frozen within 24h		Y	Y	Y	Y	Y	Y	Y	Y
Storage period (in days)	Sample	112	105	119	133	98	98	103	147
	Extraction	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Storage T° <- 18°C		Y	Y	Y	Y	Y	Y	Y	Y
Validated analytical method		Y	Y	Y	Y	Y	Y		Y
Negative controls		Y	Y	Y	Y	Y	Y	Y	Y
Considered trial		Y	Y	Y	Y	Y	Y	Y	Y
Remarks		Application history : WEEDAZ OL TL (incl.amitrole) applied in 1996, 5L/ha Mass/balance : yes	Application history : CARAZOL (incl.amitrole) applied in 1996, 12L/ha Mass/balance : yes	Application history : WEEDAZ OL TL (incl.amitrole) applied in 1996, 10L/ha Mass/balance : yes	Mass/balance : yes	Application history : AZURAL DUO (incl.amitrole) applied in 1996, 15L/ha Mass/balance : yes	Application history : WEEDAZ OL TL (incl.amitrole) applied in 1995 and 1996, 15L/ha Mass/balance : yes	Mass/balance : yes	Mass/balance : yes

Results: in all RAC grape samples, amitrole was not detected. Apart from the wine sample from trial CFP/024-1 and the must sample, in which 0.02 mg/kg and 0.01 mg/kg of amitrole was found respectively, no residue was detectable or quantifiable in the processed samples. These unexpected residues above the LOQ are not in accordance with the no-residue situation found in residue trials. Nevertheless a contamination might be suspected since several trials were previously treated with amitrole during the year before the experiment.

Considering the fact that no residue was quantifiable in the RAC, it is not possible to calculate a processing factor. Consequently it is not possible to follow the level of contamination in the processed products.

Table 7.6.3.2.-3: Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i.			: 240 g/l	Active ingredient			: amitrole					
Formulation			(e.g. WP) : SL	Crop / crop group			: wine grapes					
Commercial product			(name) : WEEDAZOL TL	Indoors / outdoors			: outdoor					
Applicant			: NUFARM SAS	Other a.s. in formulation			: synergist					
				(common name and content)			: ammonium thiocyanate 215g/L					
				Residues calculated as			: aminotriazole					
1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water (l/ha)	kg a.s./ha						
CPF024/982433 CPF/024-01 37530Limerey, France	Grapes Gamay	1) 1989 2) nr 3) 18.09.97	Spraying	1.20	311	3.74	11.03.97	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	191	grapes,wine,juice, must LOQ = 0,01 mg/kg grape n.d :LOD = 0,005 mg/kg wine,juice, must n.d :LOD = 0,00125 mg/kg Grape ¹ :RAC Grape ² : RAC before processing
		1) 1989 2) nr 3) 18.09.97	Spraying	1.20	313	3.76	11.03.97	01	grape ¹	n.d.	191	
		1) 1989 2) nr 3) 18.09.97	Spraying	2.40	306	7.35	11.03.97	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	191	
		1) 1989 2) nr 3) 18.09.97	Spraying	1.20	300	3.60	17.07.97	73	grape ¹ grape ² juice must wine	<0.01 n.d. <0.01 <0.01 <0.01	63	
		1) 1989 2) nr 3) 18.09.97	Spraying	1.20	312	3.75	19.08.97	81	grape ¹ grape ² juice must wine	n.d. n.d. <0.01 0.01 0.02	30	

Table 7.6.3.2-11 : Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i. : 240 g/l							Active ingredient : amitrole					
Formulation (e.g. WP) : SL							Crop / crop group : wine grapes					
Commercial product (name) : WEEDAZOL TL							Indoors / outdoors : outdoor					
Applicant : NUFARM SAS							Other a. s. in formulation : synergist					
							(common name and content) : ammonium thiocyanate 215g/L					
							Residues calculated as : aminotriazole					
1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water (l/ha)	kg a.s./ha						
CPF024/982433 CPF/024-02 51190, Le Mesnil, France	Grapes Chardonnay	1) 1959 2) nr 3) 25.09.97	Spraying	1,20	303	3,64	06.03.97	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	203	grapes,wine,juice, must LOQ = 0,01 mg/kg grape n.d :LOD = 0,005 mg/kg wine,juice, must n.d :LOD = 0,00125 mg/kg Grape ¹ :RAC Grape ² : RAC before processing Application history : CARAZOL (incl.amitrole) applied in 1996, 12L/ha
		1) 1959 2) nr 3) 25.09.97	Spraying	1.20	296	3.55	06.03.97	01	grape ¹	n.d.	203	
		1) 1959 2) nr 3) 25.09.97	Spraying	2.46	291	7.45	06.03.97	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	203	
		1) 1959 2) nr 3) 25.09.97	Spraying	1.20	304	3.65	03.07.97	73	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. <0.01	84	
		1) 1959 2) nr 3) 25.09.97	Spraying	1.20	303	3.64	26.08.97	81	grape ¹ grape ² juice must wine	n.d. n.d. <0.01 <0.01 <0.01	30	

Table 7.6.3.2.-5: Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i. : 240 g/l
 Formulation (e.g. WP) : SL
 Commercial product (name) : WEEDAZOL TL
 Applicant : NUFARM SAS

Active ingredient : amitrole
 Crop / crop group : wine grapes
 Indoors/outdoors : outdoor
 Other a. s. in formulation : synergist
 (common name and content) : ammonium thiocyanate 215g/L
 Residues calculated as : aminotriazole

1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water (l/ha)	kg a.s./ha						
CPF024/982433 CPF/024-03 33350, St. Pey de Castets, France	Grapes Merlot	1) 1984 2) nr 3)11.09.97	Spraying	1.20	303	3.64	10.03.93	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	185	grapes,wine,juice, must LOQ = 0,01 mg/kg grape n.d :LOD = 0,005 mg/kg wine,juice, must n.d :LOD = 0,00125 mg/kg Grape ¹ :RAC Grape ² : RAC before processing Application history : WEEDAZOL TL (incl.amitrole) applied in 1996, 10L/ha
		1) 1984 2) nr 3)11.09.97	Spraying	1.20	299	3.59	10.03.93	01	grape ¹	n.d.	185	
		1) 1984 2) nr 3)11.09.97	Spraying	1.20	294	3.53	18.06.93	71	grape ¹ grape ² juice must wine	<0.01 n.d. n.d. <0.01 <0.01	85	
		1) 1984 2) nr 3)11.09.97	Spraying	1.20	298	3.58	12.08.93	85	Grape RAC	n.d. n.d. n.d. n.d.	30	

Table 7.6.3.2-12 : Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i. : 240 g/l
 Formulation (e.g. WP) : SL
 Commercial product (name) : WEEDAZOL TL
 Applicant : NUFARM SAS

Active ingredient : amitrole
 Crop / crop group : wine grapes
 Indoors/outdoors : outdoor
 Other a. s. in formulation : synergist
 (common name and content) : ammonium thiocyanate 215g/L
 Residues calculated as : aminotriazole

1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water (l/ha)	kg a.s./ha						
CPF024/982433 CPF/024-04 30300, Beaucaire, France	Grapes Merlot	1) 1991 2) nr 3)27.08.97	Spraying	1.20	304	3.65	7.03.97	01	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	173	grapes,wine,juice, must LOQ = 0,01 mg/kg grape n.d :LOD = 0,005 mg/kg wine,juice, must n.d :LOD = 0,00125 mg/kg Grape ¹ :RAC Grape ² : RAC before processing Application history : no previous treatment with amitrole
		1) 1991 2) nr 3)27.08.97	Spraying	1.20	309	3.71	7.03.97	01	grape ¹	n.d.	173	
		1) 1991 2) nr 3)27.08.97	Spraying	1.20	300	3.60	2.06.97	71	grape ¹ grape ² juice must wine	n.d. n.d. n.d. n.d. n.d.	86	
		1) 1991 2) nr 3)27.08.97	Spraying	1.20	303	3.64	28.07.97	85	grape ¹ grape ² juice must wine	n.d. n.d. <0.01 <0.01 <0.01	30	

Table 7.6.3.2-7 Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i. : 240 g/l				Active ingredient : amitrole								
Formulation (e.g. WP) : SL				Crop / crop group : wine grapes								
Commercial product (name) : WEEDAZOL TL				Indoors / outdoors : outdoor								
Applicant : NUFARM SAS				Other a. s. in formulation : synergist								
				(common name and content) : ammonium thiocyanate 215g/L								
				Residues calculated as : aminotriazole								
1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water(l/ha)	kg a.s/ha						
CPF024/982433 CPF/024-05 30130 Pont St. Esprit France	Grapes Grenache	1) 1991 2) nr 3)18.09.97	Spraying	1.20	299	3.59	07.03.93	01	grape ¹	n.d.	195	LOQ = 0,01 mg/kg n.d :LOD = 0,005 mg/kg Grape ¹ :RAC Application history : AZURAL DUO (incl.amitrole) applied in 1996, 15L/ha
		1) 1991 2) nr 3)18.09.97	Spraying	1.20	302	3.63	07.03.93	01	grape ¹	n.d.	195	
		1) 1991 2) nr 3)18.09.97	Spraying	1.20	298	3.58	06.06.93	71	grape ¹	n.d.	104	
		1) 1991 2) nr 3)18.09.97	Spraying	1.20	298	3.58	19.08.93	85	grape ¹	n.d.	30	
CPF024/982433 CPF/024-06 68000, Colmar, France	Grapes Riesling	1) 1987 2) nr 3)02.10.97	Spraying	1.20	301	3.62	05.03.93	01	grape ¹	n.d.	211	LOQ = 0,01 mg/kg n.d :LOD = 0,005 mg/kg Grape ¹ :RAC Application history : WEEDAZOL TL (incl.amitrole) applied in 1995 and 1996, 15L/ha
		1) 1987 2) nr 3)02.10.97	Spraying	1.20	305	3.66	05.03.93	01	grape ¹	n.d.	211	
		1) 1987 2) nr 3)02.10.97	Spraying	1.20	299	3.59	25.06.93	71	grape ¹	n.d.	99	
		1) 1987 2) nr 3)02.10.97	Spraying	1.20	300	3.60	02.09.93	81	grape ¹	<0.01	30	

Table 7.6.3.2-8 : Residues data summary from supervised trials (summary) (Wilson study on grape)

Content of a.i. : 240 g/l				Active ingredient : amitrole								
Formulation (e.g. WP) : SL				Crop / crop group : wine grapes								
Commercial product (name) : WEEDAZOL TL				Indoors / outdoors : outdoor								
Applicant : NUFARM SAS				Other a. s. in formulation : synergist								
				(common name and content) : ammonium thiocyanate 215g/L								
				Residues calculated as : aminotriazole								
1	2	3	4	5			6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	PHI (days) (f)	Remarks (g)
				kg a.s./hL	Water(l/ha)	kg a.s/ha						
CPF024/982433 CPF/024-07 Sotés, Sapin	Grapes Tempranillo	1) 1989 2) nr 3)19.09.97	Spraying	1.20	309	3.71	27.02.93	01	grape ¹	n.d.	203	LOQ = 0,01 mg/kg n.d :LOD = 0,005 mg/kg Grape ¹ :RAC Application history : no previous treatment with amitrole
		1) 1989 2) nr 3)19.09.97	Spraying	1.20	300	3.60	27.02.93	01	grape ¹	n.d.	203	
		1) 1989 2) nr 3)19.09.97	Spraying	1.20	295	3.54	05.06.93	71	grape ¹	n.d.	105	
		1) 1989 2) nr 3)19.09.97	Spraying	1.20	305	3.66	19.08.93	82	grape ¹	n.d.	30	
CPF024/982433 CPF/024-08 Jerez, Spain	Grapes Palomino	1) 1969 2) nr 3)14.08.97	Spraying	1.20	310	3.72	26.02.93	01	grape ¹	n.d.	169	LOQ = 0,01 mg/kg n.d :LOD = 0,005 mg/kg Grape ¹ :RAC Application history : no previous treatment with amitrole
		1) 1969 2) nr 3)14.08.97	Spraying	1.20	307	3.69	26.02.93	01	grape ¹	n.d.	169	
		1) 1969 2) nr 3)14.08.97	Spraying	1.20	312	3.75	07.05.93	74	grape ¹	n.d.	99	
		1) 1969 2) nr 3)14.08.97	Spraying	1.20	308	3.70	15.07.93	82-83	grape ¹	n.d.	30	

B.7.6.3.3. Additional residue study on in grape – Amic (2012)

Study	KIIA 6.3.1/02 - Amitrole: residue study on grapes in France in 2011.
Reference	Amic. (2012)
Date performed	2011
Test facility	Eurofins ADME BIOANALYSE
Report reference	No: S11-01150
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	No
GLP	Yes
Test material	Commercial product CA-2735 with 229 g/l of amitrole
Study acceptable	Yes

Material and method : the purpose of this study was to determine the amount of residue of amitrole and its metabolites triazolyalanine and aminotriazolyalanine in samples at normal commercial harvest after one application of the test item (CA2735) targeted at 13L/ha equivalent to 3000 g a.i./ha. Grape specimens were collected at normal commercial harvest. Test item was applied once per plot by plot sprayer on the soil. Four trials were conducted in France. The principle of the analytical method used is as follows: residues of amitrole and its metabolites triazolyalanine and aminotriazolyalanine were extracted by homogenization with acetone/ultra pure water (75/25, v/v) mixture. After filtration an aliquot was clean-up by liquid-liquid partition with dichloromethane and then hexane. The aqueous phase was acidified and purified over an MCX cartridge (500 mg / 6 mL). After evaporation, the dry extract was dissolved in ultra pure water and then analysed by LC-LC-MS/MS (for amitrole) and LC-MS/MS (for the metabolites) with electropositive and electronegative ion spray. The limit of quantification (LOQ) for amitrole and its metabolites triazolyalanine and aminotriazolyalanine is 0.01 mg/kg.

Table 7.6.3.3-1 : Summary of global information (Amic study, residue trial on grape)

Comparative trials (between formulations, with and w/o adjuvant/safener/synergist)	No but the product also contains the synergist ammonium thiocyanate 215g/L
Number of applications	1
Dose (g as/ha)	3000 : 13 L/ha of product CA 2735 SL containing 229 g/l of active substance and 215g/L of the synergist ammonium thiocyanate
Mode of application	Soil application by spraying
PHI (days) and/or growth stage (BBCH)	Application at 86-114 days before harvest
Analytical method (Code +Type)	Method AGR/MOA/AMT-8 : aminotriazole ; triazolyalanine, aminotriazolyalanine (LC-MS/MS)
LoQ (mg/kg)	0,01 for each: aminotriazole, triazolyalanine and aminotriazolyalanine

Table 7.6.3.3-13 : Details about the trials (Amic study, residue trial on grape)

N° Trial	S11-0115-01	S11-0115-02	S11-0115-03	S11-0115-04
North/South/Indoor	N	N	S	S
Decline (D)/Harvest (H) trial?	H	H	H	H
Formulation	SL	SL	SL	SL
Equivalence between formulations	Y	Y	Y	Y
Accordance with intended GAP (1)	N	N	N	N
Correct sampling	Y	Y	Y	Y
Samples frozen within 24h	Y	Y	Y	Y
Storage period (in days)	Sample (2)	September to November 2011		
	Extract (3)	Max.11 days		
Storage T° <-18°C	Y	Y	Y	Y
Validated analytical method	Y	Y	Y	Y
Negative controls	Y	Y	Y	Y
Considered trial	Y	Y	Y	Y
Remarks (1)			Applic-early GS BBCH55	

Result all residues were below the LOQ of 0.01 mg/kg for each amitrole and metabolites triazolyalanine and aminotriazolyalanine.

Table 7.6.3.3-3 : Residues data summary from supervised trials (summary) (Amic study on grape)

(Application on agricultural and horticultural crops)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : CA 2735

Applicant : NUFARM SAS

Active ingredient : amitrole

Crop / crop group : wine grapes

Indoors / outdoors : outdoors

Other a. s. in formulation : synergist

(common name and content) : ammonium thiocyanate 215g/L

Residues calculated as : aminotriazole and triazolylalanine and
aminotriazolylalanine

1	2	3	4	5			6	7	8	9			10	11
Report No. Trial No. Location (region)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s) or no of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed (a)	Residues (mg/kg)			PHI (days) (f)	Remarks (g)
				kg a.s./ha	Water (l/ha)	kg a.s./hl				Amitrole	Triazolyl alanine	Aminotri azolylala nine		
S11-01150/01 67140 Gertwiller Bas- Rhin	Grapes Pinot blanc Auxerrois	1) 1984 2) nr 3) 15.09.11	Soil spraying	3,013	301	1,00	07.06.11	71	Grapes	<0.01	<0.01	<0.01	100	LOQ = 0,01 mg/kg
S11-01150/02 49540 Togné, Maine et Loire	Grapes Gamay	1) 15.02.00 2) 20 to 30.05.11 3) 07.09.11	Soil spraying	3,297	240	1,37	08.06.11	79	Grapes	<0.01	<0.01	<0.01	91	
S11-01150/03 82700 Bourret Tarn et Garonne	Grapes Tanat	1) 2005 2) nr 3) 05.09.11	Soil spraying	3,294	439	0,75	13.05.11	55	Grapes	<0.01	<0.01	<0.01	115	
S11-01150/04 66200 Elne, Pyrénées Orientales	Grapes Cabernet Sauvignon	1) 1995 2) nr 3) 05.09.11	Soil spraying	2,850	380	0,75	10.06.11	73-75	Grapes	<0.01	<0.01	<0.01	87	

Remarks:

(a) According to EEC and Codex class classifications (both) should be used

(b) Only if relevant

(c) High or low volume spraying, spreading, dusting etc., overall broadcast, type of equipment used must be indicated

(d) Year must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

(f) Minimum number of days after application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

B.7.6.4. Supervised trials on olives**Table 7.6.4.-1:** Overview of the uses of amitrole on olives in EU

Crop	Type of GAP	Number of applic.	Application rate per treatment (precise unit)	Interval between application	Growth stage at last application	PHI days
Olive	EU S (PT)	1	960-2580 g a.s/ha Soil treatment spraying	60 days	In olives tree, do not apply after the falling of the fruit.	n.a
	EU AIR II (S)	1-2	900-1375 g a.s/ha	2-3 months	BBCH 92-79 – Broadcast spraying under the row or on localised areas with tractor mounted boom. Application in spring and autumn. CA2735 is applied either once per year at 2977 gai/ha (or several times at lower rates but respecting the maximum application rate /year of 3000 gai/ha/year). A split application technique is possible at lower rates especially in case of mixture with foliar or residual herbicide according to the crop.	35

B.7.6.4.1. Studies submitted in first monograph

The use was not studied during the first inclusion

B.7.6.4.2. Residue study on olive – Anonymous (2000)

Study	KIIA 6.3.3/03 - Analisis de residuos de 3-amino-1,2,4-triazole en aceitunas tratadas con ETIZOL TL por HPLC-fluorescencia.
Reference	Anonymous. (2000a)
Date performed	1998
Test facility	Laboratory Agro-alimentari, Cabrils (E)
Report reference	No: report n° 401991S/1N- 401992S/1N- 401993S/1N- 401995S/2N- 401001B/N- 401001C/N- 401001S/1N
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	Yes
GLP	No
Test material	Commercial product ETIZOL TL with 240g/L of amitrole
Study acceptable	No

Study	KIIA 6.3.3/04 - Analisis de residuos de 3-amino-1,2,4-triazole en aceitunas tratadas con MAXATA TL por HPLC-fluorescencia.
Reference	Anonymous. (2000b)
Date performed	1998
Test facility	Laboratory Agro-alimentari, Cabrils (E)
Report reference	No: 401991S/2N- 401992S/2N- 401993S/2N- 401995S/3N- 401002B/N- 401002C/N- 401002U/
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	Yes
GLP	No
Test material	Commercial product MAXATA with 860g/Kg of amitrole
Study acceptable	No

Table 7.6.4.2-14 Summary of global information (Anonymous, residue trial on olive)

Comparative trials (between formulations, with and w/o adjuvant/safener/synergist)	Yes, ETIZOL TL and not MAXATA formulations. ETIZOL contains also the synergist ammonium thiocyanate : 210g/L
Number of applications	1
Dose (g as/ha)	1920g/ha of amitrole with the product ETIZOL 3440 of amitrole with the product MAXATA
Mode of application	Soil application by spraying
PHI (days) and/or growth stage (BBCH)	Harvest at 0, 21, 28, 35 days after the application (ETIZOL) Harvest at 0, 21, 28 days after the application (MAXATA) Harvest of olives fallen on the floor and directly in the tree
Analytical method (Code +Type)	HPLC/F PNT030074
LoQ (mg/kg)	Not validated

Details about the trials: 5 trials were conducted in Spain, in the Cataluña region and the Andalucía region, using MAXATA (SG, 860 g/kg) and ETIZOL TL. Olives were sampled at random from the trees with PHIs of 0, 21, 28 or 35 days. Olives present on the soil at the treatment were also sampled from the soil with same PHIs.

Nevertheless the analytical method used for the determination of amitrole residues in both reports was not validated. Trials on olives cannot be taken into account.

B.7.6.4.3. Residue study on olive – Amic (2011)

Study	KIIA 6.3.3/01 - Amitrole – Residue Study on Olives in Spain in 2010
Reference	Amic.S (2011)
Date performed	2010
Test facility	Eurofins
Report reference	No: S10-03088
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	No
GLP	Yes
Test material	ETIZOL TL with 240g/L of amitrole
Study acceptable	Yes

Material and method: the purpose of this study was to determine the amount of residue of amitrole and its metabolites triazolyllalanine and aminotriazolyllalanine in table and processed olive samples at normal commercial harvest after 1 soil application of the test item (ETIZOL TL) targeted at 2kg a.i./ha. Different pre-harvest intervals were tested.

Olive specimens were collected on different sub-plots after 21 or 28 or 35 days after application. Test item was applied once per sub-plots by plot sprayer on the soil. 4 trials were conducted in Spain.

Tables' olives were sampled by hand. Processing olives were sampled with a net placed under the tree on the soil after shaking with a stick. Olives were then collected from the net. Olives were stored in frozen condition during transport and before extraction/analysis.

The principle of the analytical method used is as follows: residues of amitrole and its metabolites triazolyllalanine and aminotriazole alanine were extracted by shaking with a 75/25 v/v acetone/ultra pure water mixture. After filtration, an aliquot was clean-up by liquid-liquid partition with dichloromethane and then hexane. The aqueous phase was acidified and purified over an MCX cartridge (500mg/6mL). After evaporation, the dry extract was dissolved in ultra pure water and then analysed by LC-LC-MS/MS (for amitrole) and LC-MS/MS (for the metabolites) with electropositive and electronegative spray. The limit of quantification for amitrole and its metabolites is for each 0.01mg/kg.

Table 7.6.4.3-1 : Summary of global information (Amic study, residue trial on olive)

Comparative trials (between formulations, with and adjuvant/safener/synergist)	No but the product also contains the synergist ammonium thiocyanate 210g/L
Number of applications	1
Dose (g as/ha)	2000
Mode of application	Spray
PHI (days) and/or growth stage (BBCH)	PHI: 21, 28 and 35 (BBCH at application: 75-89)
Analytical method (Code +Type)	LC-LC-MS/MS (for amitrole) and LC-MS/MS (for the metabolites: triazolyllalanine + aminotriazolyllalanine) with electropositive and electronegative ion spray
LoQ (mg/kg)	0.01

Table 7.6.4.3-2 : Details about the trials (Amic study, residue trial on olive)

N° Trial	S10-03088-01	S10-03088-02	S10-03088-03	S10-03088-04
North/South/Indoor	S	S	S	S
Decline(D)/Harvest(H) trial?	D	D	D	D
Formulation	SL	SL	SL	SL
Equivalence between formulations	Y	Y	Y	Y
Accordance with intended GAP	Y	Y	Y	Y
Correct sampling	Y	Y	Y	Y
Samples frozen within 24h	Y	Y	Y	Y
Storage period (in days)	< 180			
Sample				
Extract	< 6			
Storage T° <-18°C	Y	Y	Y	Y
Validated analytical method	Y	Y	Y	Y
Negative controls	Y	Y	Y	Y
Considered trial	Y	Y	Y	Y
Remarks	-	-	-	-

Results: all results (samples collected by hand or with a net) were below the LOQ for each aminotriazole and its metabolite triazolyllalanine and aminotriazolyllalanine.

Table 7.6.4.3-15 : Residues data summary from supervised trials (summary) (Amic study on olive)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : ETIZOL TL

Applicant : NUFARM SAS

Active ingredient : amitrole

Crop / crop group : olives

Indoors / outdoors : outdoors

Other a. s. in formulation
(common name and content) : ammonium thiocyanate 210g/LResidues calculated as : aminotriazole and
triazolylalanine and
aminotriazolylalanine

1	2	3	4	5			6	7	8	9			10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no of treatment(s)	Growth stage at first treatment or date	Portion analysed (a)	Residues (mg/kg)			PHI (days) (f)	Remarks (g)
				kg a.s./hl	Water (l/ha)	kg a.s./ha				Amitrole	Triazolyl alanine	Aminotri- azolylala- nine		
S10-03088-01 14546 Santaella Cordoba, Spain	Olive marteno	1) not reported 2) not reported 3)15.10.2010	soil spraying	0.667	314,3	2.096	10.09.2010	78	olive ¹	<0.01	<0.01	<0.01	35	¹ :collected by hand ² :collected on a net
		1) not reported 2) not reported 3)15.10.2010	soil spraying	0.666	305.2	2.033	17.09.2010	78	olive ¹	<0.01	<0.01	<0.01	28	
		1) not reported 2) not reported 3)15.10.2010	soil spraying	0.667	308.7	2.058	24.09.2010	78	olive ¹	<0.01	<0.01	<0.01	21	
		1)not reported 2) not reported 3)02.12.2010	soil spraying	0.651	315.9	2.058	27.10.2010	81	olive ²	<0.01	<0.01	<0.01	35	
		1)not reported 2) not reported 3)02.12.2010	soil spraying	0.664	315.1	2.105	03.11.2010	85	olive ²	<0.01	<0.01	<0.01	28	
		1)not reported 2) not reported 3)02.12.2010	soil spraying	0.670	315.5	2.114	10.11.2010	85	olive ²	<0.01	<0.01	<0.01	21	

Table 7.6.4.3-4 : Residues data summary from supervised trials (summary) (Amic study on olive)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : ETIZOL TL

Applicant : NUFARM SAS

Active ingredient : amitrole

Crop / crop group : olives

Indoors / outdoors : outdoors

Other a. s. in formulation

(common name and content) : ammonium thiocyanate 210g/L

Residues calculated as : aminotriazole and triazolylalanine
and aminotriazolylalanine

1	2	3	4	5			6	7	8	9			10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no of treatment(s)	Growth stage at last treatment or date	Portion analysed (a)	Residues (mg/kg)			PHI (days) (f)	Remarks (g)
				kg a.s./hl	Water (l/ha)	kg a.s./ha				Amitrole	Triazolyl alanine	Aminotri azolylala nine		
S10-03088-02 41550 Aguadulce Sevilla, Spain	Olive marteno	1) not reported 2) not reported 3)15.10.2010	soil spraying	0.666	290.6	1.937	10.09.2010	78	olive ¹	<0.01	<0.01	<0.01	35	¹ :collected by hand ² :collected on a net
		1) not reported 2) not reported 3)15.10.2010	soil spraying	0.667	301.4	2.009	12.09.2010	78	olive ¹	<0.01	<0.01	<0.01	28	
		1) not reported 2) not reported 3)15.10.2010	soil spraying	0.666	300.4	2.002	24.09.2010	78	olive ¹	<0.01	<0.01	<0.01	21	
		1)not reported 2) not reported 3)25.11.2010	soil spraying	0.666	311.8	2.078	21.10.2010	81	olive ²	<0.01	<0.01	<0.01	35	
		1)not reported 2) not reported 3)25.11.2010	soil spraying	0.666	304.5	2.029	28.10.2010	81	olive ²	<0.01	<0.01	<0.01	28	
		1)not reported 2) not reported 3)25.11.2010	soil spraying	0.666	299.3	1.995	04.11.2010	85	olive ²	<0.01	<0.01	<0.01	21	

Table 7.6.4.3-5 : Residues data summary from supervised trials (summary) (Amic study on olive)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : ETIZOL TL

Applicant : NUFARM SAS

Active ingredient : amitrole

Crop / crop group : olives

Indoors / outdoors : outdoors

Other a. s. in formulation
(common name and content) : ammonium thiocyanate 210g/LResidues calculated as : aminotriazole and
triazolylalanine and
aminotriazolylalanine

1	2	3	4	5			6	7	8	9			10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no of treatment(s)	Growth stage at last treatment or date	Portion analysed (a)	Residues (mg/kg)			PHI (days) (f)	Remarks (g)
				kg a.s./hl	Water (l/ha)	kg a.s./ha				Amitrole	Triazolyl alanine	Aminotri azolylala nine		
S10-03088-03 50280 Calatorao, Zaragoza, Spain	Olive arbequina	1)not reported 2) not reported 3)25.10.2010	soil spraying	0.667	315.3	2.103	20.09.2010	75	olive ¹	<0.01	<0.01	<0.01	35	¹ :collected by hand ² :collected on a net
		1)not reported 2) not reported 3)25.10.2010	soil spraying	0.666	304.2	2.027	27.09.2010	76	olive ¹	<0.01	<0.01	<0.01	28	
		1)not reported 2) not reported 3)25.10.2010	soil spraying	0.666	294.5	1.962	04.09.2010	77	olive ¹	<0.01	<0.01	<0.01	21	
		1)not reported 2) not reported 3)14.12.2010	soil spraying	0.667	301.4	2.009	11.11.2010	85	olive ²	<0.01	<0.01	<0.01	33	
		1)not reported 2) not reported 3)14.12.2010	soil spraying	0.499	398.6	1.991	16.11.2010	85-89	olive ²	<0.01	<0.01	<0.01	28	
		1)not reported 2) not reported 3)14.12.2010	soil spraying	0.667	322.2	2.148	23.11.2010	85-89	olive ²	<0.01	<0.01	<0.01	21	

Table 7.6.4.3-6 : Residues data summary from supervised trials (summary) (Amic study on olive)

Notifier: NUFARM SAS

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : ETIZOL TL

Applicant : NUFARM SAS

Active ingredient : amitrole

Crop / crop group : olives

Indoors / outdoors : outdoors

Other a. s. in formulation
(common name and content) : ammonium thiocyanate 210g/LResidues calculated as : aminotriazole and
triazolylalanine and
aminotriazolylalanine

1	2	3	4	5			6	7	8	9			10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no of treatment(s)	Growth stage at last treatment or date	Portion analysed (a)	Residues (mg/kg)			PHI (days) (f)	Remarks (g)
				kg a.s./hl	Water (l/ha)	kg a.s./ha				Amitrole	Triazolyl alanine	Aminotri azolylala nine		
S10-03088-04 46625 Fontanars dels Aforins, Valencia, Spain	Olive arbequina	1)not reported 2) not reported 3)09.11.2010	soil spraying	0.666	307.5	2.050	05.10.2010	79	olive ¹	<0.01	<0.01	<0.01	35	¹ :collected by hand ² :collected on a net
		1)not reported 2) not reported 3)09.11.2010	soil spraying	0.666	290.2	1.934	14.10.2010	80	olive ¹	<0.01	<0.01	<0.01	26	
		1)not reported 2) not reported 3)09.11.2010	soil spraying	0.667	302.3	2.016	19.10.2010	80	olive ¹	<0.01	<0.01	<0.01	21	
		1)not reported 2) not reported 3)24.11.2010	soil spraying	0.667	313.1	2.087	25.10.2010	81	olive ²	<0.01	<0.01	<0.01	30	
		1)not reported 2) not reported 3)24.11.2010	soil spraying	0.667	309.5	2.063	02.11.2010	85	olive ²	<0.01	<0.01	<0.01	22	
		1)not reported 2) not reported 3)24.11.2010	soil spraying	0.667	286.5	1.910	10.11.2010	85	olive ²	<0.01	<0.01	<0.01	14	

(a) According to EEC and Codex class classifications (both) should be used

(b) Only if relevant

(c) High or low volume spraying, spreading, dusting etc., overall broadcast, type of equipment used must be indicated

(d) Year must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

(f) Minimum number of days after application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

B.7.6.4.4. Residue trial in olive – Amic (2011)

Study	KIIA 6.3.3/02 - Amitrole – residue exploratory trial on processing olives in Spain in 2010.
Reference	Amic.S (2011)
Date performed	2010
Test facility	Eurofins
Report reference	No: S10-03541N
Guideline(s)	EEC 7029/VI/95 rev. 5 Appendix B (EU): General recommendation for the design, preparation and realization of residue trials
Deviations from the guideline	No
GLP	Yes
Test material	ETIZOL TL with 240g/L of amitrole
Study acceptable	Yes

Materials and methods : The propose of this study was to determine the amount of residue of amitrole in processing olive samples at normal commercial harvest after 1 application of the test item (Etizol TZ) targeted at 2000g as/ha at different pre-harvest intervals and tree shaking-sampling intervals. Olive specimens were collected on different sub-plots after 6, 12, 20, 27 and 33 day after application. Test item was applied one per sub-plot by plot sprayer on the soil. One trial was conducted in Spain.

The method referenced as AGR/MOA/AMT-2 at Eurofins/ADME BIOANALYSES has been used for the analysis of amitrole in olives: residues of amitrole were extracted by shaking with a 75/25 v/v acetone/ultra pure water mixture. After filtration, an aliquot was clean-up by liquid-liquid partition with dichloromethane and then hexane. The aqueous phase was acidified and purified over an MCX cartridge (500mg/6mL). After evaporation, the dry extract was dissolved in ultra pure water and then analyzed by LC-LC-MS/MS (for amitrole) and LC-MS/MS (for metabolites) with electropositive and electronegative ion spray. The limit of quantification for amitrole is 0.01mg/kg.

Table 7.6.4.4.-1 : Summary of global information (Amic study, residue trial on olive)

Comparative trials (between formulations, with and adjuvant/safener/synergist)	No but the product also contains the synergist ammonium thiocyanate 210g/L
Number of applications	1
Dose (g as/ha)	c.a. 2000
Mode of application	soil spraying (BBCH at application: 81)
PHI (days) and/or growth stage (BBCH)	PHI: 6,12, 20, 27, 33 (BBCH 81-85 or 85-89)
Analytical method (Code +Type)	AGR/MOA/AMT-2: LC-LC-MS/MS (for amitrole) and LC-MS/MS (for metabolites) with electropositive and electronegative ion spray
LoQ (mg/kg)	0.01

Table 7.6.4.4-2 : Details about the trials (Amic study, residue trial on olive)

N° Trial	S10-03541N-01 (P2a, P2b, P3a, P3b, P4a, P4b, P5a, P5b)	
North/South/Indoor	S (Spain)	
Decline (D)/Harvest (H) trial?	H	
Formulation	SL	
Equivalence between formulations	Y	
Accordance with intended GAP	Y	
Correct sampling	Y	
Samples frozen within 24h	Y	
Storage period (in days)	Sample	< 120
	Extract	< 2
Storage T°<-18°C	Y	
Validated analytical method	Y	
Negative controls	Y	
Considered trial	Y	
Remarks	-	

Results: no residues of amitrole were found in olive samples were detected at or above the limit of quantification (0.01mg/kg) when the application-tree shaking interval is 0-20 days; the tree shaking-sampling interval is 6-14 days and the application-sampling interval is 6-33 days.

No residues of amitrole in the control processing olive sample were detected at or above the limit of quantification.

Table 7.6.4.4.-3: Residues data summary from supervised trials (summary) (Amic study on olive)

Notifier NUFARM

Content of a.i. (g/kg or g/l) : 229 g/l

Formulation (e.g. WP) : SL

Commercial product (name) : ETIZOL TL

Active ingredient : amitrole

Crop / crop group : olives

Indoors / outdoors : outdoors

Other a. s. in formulation : ammonium thiocyanate 210g/L

Residues calculated as : aminotriazole and triazolylalanine
and aminotriazolylalanine

1	2	3	4	5	6	7	8	9	10	11
Report No. Trial No. Location (region)	Commodity/ Variety (a)	Date of 1.Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment kg a.s./hl Water (l/ha) kg a.s./ha	Dates of treatment(s) or no of treatment(s)	Growth stage at last treat. or date	Portion analysed (a)	Residues (mg/kg) Aminotriazole Triazolylalanine Aminotriazolylalanine	PHI (days) (f)	Remarks (g)
S10-03541N-01 29400 Ronda Andalucia, Spain	Olive malvena	1) not reported 2) not reported 3)05/11/2010	soil spraying	0.671 294 1.974	30/10/2010	81	olive ¹ BBCH 81-85	<0.01 <0.01 <0.01	6 DALA (² 0)	¹ : collected on a net ² : days before tree shaking DALA : days after last application
		1) not reported 2) not reported 3)11/11/2010	soil spraying	0.672 301 2.022	30/10/2010	81	olive ¹ BBCH 81-85	<0.01 <0.01 <0.01	12 DALA (² 0)	
		1)not reported 2) not reported 3)11/11/2010	soil spraying	0.670 304 2.038	30/10/2010	81	olive ¹ BBCH 81-85	<0.01 <0.01 <0.01	12 DALA (² 6)	
		1)not reported 2) not reported 3)19/11/2010	soil spraying	0.671 300 2.013	30/10/2010	81	olive ¹ BBCH 81-85	<0.01 <0.01 <0.01	20 DALA (² 6)	
		1)not reported 2) not reported 3)19/11/2010	soil spraying	0.672 297 1.997	30/10/2010	81	olive ¹ BBCH 81-85	<0.01 <0.01 <0.01	20 DALA (² 12)	
		1)not reported 2) not reported 3)26/11/2010	soil spraying	0.671 307 2.061	30/10/2010	81	olive ¹ BBCH 85-89	<0.01 <0.01 <0.01	27 DALA (² 12)	
		1)not reported 2) not reported 3)26/11/2010	soil spraying	0.670 290 1.944	30/10/2010	81	olive ¹ BBCH 85-89	<0.01 <0.01 <0.01	27 DALA (² 20)	
		1)not reported 2) not reported 3)02/12/2010	soil spraying	0.674 302 2.027	30/10/2010	81	olive ¹ BBCH 85-89	<0.01 <0.01 <0.01	33 DALA (² 20)	

(a) According to EEC and Codex class classifications (both) should be used

(b) Only if relevant

(c) High or low volume spraying, spreading, dusting etc. overall broadcast, type of equipment used must be indicated

(d) Year must be indicated

(e) BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

(f) Minimum number of days after application (Label pre-harvest interval, PHI, underline)

(g) Remarks may include: Climatic conditions; Reference to analytical method; Information concerning the metabolites included, the method of storage, storage stability, analysis date

B.7.6.5. Summary on available residue data for intended uses on orchard, grape and olive

Following EFSA requirement stated in its reasoned opinion under article 12, storage stability studies were provided in apples, grapes and olives. These studies demonstrated that amitrole was stable in high water, acid and lipid content matrices under frozen conditions. Additionally, storage stability of triazolylalanine and aminotriazolylalanine in grapes and olives were also demonstrated.

Since the first inclusion, new residue trials were provided on apple, grape and olive. For information, most of these trials were also realized in presence of the synergist ammonium thiocyanate and demonstrated that residues are below the LOQ in fruits (apple, olive and grape). This assessment goes on the same way than the EFSA reasoned opinion about the review of the existing maximum residue levels (MRLs) for amitrole according to Article 12 of Regulation (EC) No 396/2005 which is also based on previous results from residue trials on apple, pear, peach and grape provided in the initial monograph.

Additionally new residue results are also available for triazolylalanine and aminotriazolylalanine in grapes and olives which also demonstrate a no residue situation. Consequently, the need to include these metabolites in the residue definition is not considered necessary in regard to intended uses on orchards, grapes and olives. Trials on apple with detailed results on triazolylalanine and aminotriazolylalanine would also be soon available but currently in progress (Amic.S, 2013).

Pursuant to the EU guideline "Comparability, extrapolation, group tolerances and data requirements"⁹, since the no residue situation is compliant with the MRL in force of 0.01mg/kg, the data package is considered acceptable for orchards (citrus fruits, pome and stone fruits and tree nuts).

Residue trials with olives also demonstrate a no residue situation which is compliant with the MRL in force of 0.05mg/kg. Pursuant to the same guideline, in this situation, a full data package is not required and available data on olives are considered acceptable.

Table 7.6.5-16 Summary of residue results on orchard, grapes and olives

Zone	Number of trials supporting the GAP	Residues (mg/kg)	STMR	HR	Comments	
Enforcement and risk assessment (residue definition : amitrole)						
Orchards						
GAP : 1 x 1.2-3kg a.i/ha – 35 day PHI						
Northern EU	6	Apples and pear	<0.01x4; <0.02 x 2	0.01	0.01	Dataset on apples(5) and pear(1) with 1-2 application (total application comprised between 8-10kg/ha) PHI comprised between 4 and 42 days 13 others trials : dataset on apples (9), pears(4) in north with 1-4 application (total application comprised between 5.8-15kg/ha) PHI comprised between 49 and 111 days and <LOQ
Southern EU	9	Apples and pear and peach	<0.01 x 9	0.01	0.01	Combined dataset on apples(4), pear(1); and peaches(4) with 1-4 application (total application comprised between 5.8-11.7kg/ha) PHI comprised between 37 and 111 days
Grapes						
GAP : 1 x 1.2-3kg a.i/ha – 42 day PHI						
Northern EU	11	Fruit	<0.01 x 5; <0.02 x 6	0.01	0.01	with 1-2 application (total application comprised between 3.6-8kg/ha) PHI comprised between 28 and 43 days
Southern EU	9	Fruit	<0.01 x 5; <0.025 x 4	0.01	0.01	with 1 application (total application comprised between 2.9-3.7kg/ha) PHI comprised between 15 and 30 days
Olives						
GAP : 1 x 0.9-3kg a.i/ha – 35 day PHI						
Southern EU	5	Fruit	<0.01x 5	0.01	0.01	with 1 application (total application comprised between 1.9-2.1kg/ha) PHI comprised between 6 and 35 days

Commission of the European Communities, Directorate General for Health and Consumer Protection, working document Doc. 7525/VI/95-rev.9 – table 4 : extrapolation of active substance used early in the growing season (last application before consumable parts of the crop have started to form)

B.7.7. EFFECTS OF INDUSTRIAL PROCESSING AND/OR HOUSEHOLD PREPARATION (ANNEX IIA 6.5, ANNEX IIIA 8.4)

As quantifiable residues of amitrole are not expected in the treated crops, there is no need to investigate the effect of industrial and/or household processing. Although not required, one study investigating the magnitude of residues in grapes, juice, must and wine (white and red) was provided (Wilson, 1999, previously described in B.7.6.3.2). Several scenarios of use were assessed in those trials. Early application in the season was first considered with a treatment performed at BBCH 01. At harvest, no amitrole residue was detected either in grapes or in the processed fractions. Two other periods of treatment were investigated, BBCH71-73 (fruit development) and BBCH81-85 (ripening of berries). Residues in grapes were always lower than the LOQ (0.01 mg/kg). In only one trial (CFP/024-01), despite a residue level in grape below the LOQ, 0.01 mg/kg and 0.02 mg/kg were detected in the corresponding must and wine respectively. Nevertheless, in this trial, contamination by previous treatments with products containing amitrole is highly suspected.

No robust processing factors could be derived since residues were below the LOQ in both raw and processed commodity.

B.7.8. LIVESTOCK FEEDING STUDIES (ANNEX IIA 6.4, ANNEX IIIA 8.3)

Since the first inclusion, studies on nature or magnitude of residues in commodities of animal origin are not available, nor required. Following the estimation of the dietary burden arising from the intended uses of amitrole, MRLs for livestock products are not required because livestock is not expected to be exposed to significant levels of amitrole residues.

B.7.9. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS (ANNEX IIA 6.6, ANNEX IIIA 8.5)

Orchards, grapes and olives are not expected to be grown in rotation. Further investigation of residues in rotational crops is therefore not required. In addition, it is noted that, after 112 days, less than 0.4% of the applied dose (20kg a.i./ha equivalent) of ¹⁴C-amintriazole could be extracted from the top 5 cm of soil and corresponding concentrations of extractable ¹⁴C-amintriazole in the top 5 cm of soil were less than 0.1mg/kg.

Further information would be required in the future if additional uses are intended on rotated crops and when at EU level a full assessment report about triazoles derivatives metabolites will be available.

B.7.10. PROPOSED PRE-HARVEST INTERVALS FOR ENVISAGED USES, OR WITH HOLDING PERIODS, IN THE CASE OF POST-HARVEST USES (ANNEX IIA 6.8, ANNEX IIIA 8.7)

CROP	GAP
Orchards (citrus fruits, pome and stone fruits, assorted fruits- edible or inedible peel, tree nuts)	1.2-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year with BBCH growth stage between 94-75 and 35 day PHI
Grapes (table and vine)	1.2-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year with BBCH growth stage between 92-79 and 42 day PHI
Olives (table and oil production)	0.9-1.375 kg a.i./ha, 1-2 application or maximum application rate /year of 2750 g a.i./ha/year in spring and autumn and 35 day PHI
Non crop uses (impermeable and permeable surfaces: road, railways)	1.8-3 kg a.i./ha, 1 application or maximum application rate /year of 3000 g a.i./ha/year no edible commodity : no PHI

B.7.11. MRLs IN EU MEMBER STATES (ANNEX IIIA 12.2)

Residues are in accordance and comply with MRLs in force for orchards, table and wine grape at 0.01 mg/kg and table and olives for oil production at 0.05 mg/kg (149/2008/EC). Residues also comply with in force CXL at 0.05* mg/kg for pome fruits and stone fruits and 0.05 mg/kg for table grape and wine grape.

B.7.12. PROPOSED EU MRLs AND JUSTIFICATION FOR THE ACCEPTABILITY OF THOSE MRLs (ANNEX IIA 6.7, ANNEX IIIA 8.6)

Concerning MRL evolution, this addendum goes on the same way than the previous EFSA reasoned opinion (EFSA 2012) about the review of the existing maximum residue levels (MRLs) for amitrole according to Article 12 of Regulation (EC) No 396/2005.

B.7.13. ESTIMATES OF POTENTIAL AND ACTUAL DIETARY EXPOSURE THROUGH DIET AND OTHER MEANS (ANNEX IIA 6.9, ANNEX IIIA 8.8)**B.7.13.1. Risk assessment for chronic exposure through diet**

There is no evolution or modification since the last assessment provided by EFSA in its reasoned opinion under the article 12 of the Regulation (EC) No 396/2005. The same ADI was considered in the reasoned opinion (EFSA 2012). **ADI was set at 0.001 mg/kg bw/d** based on thyroid effects observed in the 90-day rat studies and by using a safety factor of 100 (Review Report (6839/VI/97-final 22 march 2001)).

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Table 7.13.1.-1 EU scenario including MRL of intended uses with amitrole

AMITROLE

Status of the active substance:	AIR II	Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	0.01
Toxicological end points			
ADI (mg/kg bw/day):	0.001	ARFD (mg/kg bw):	0.015
Source of ADI:	AIR II	Source of ARFD:	AIR II
Year of evaluation:	2012	Year of evaluation:	2012

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL). The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

		TMDI (range) in % of ADI minimum - maximum							
		2		20					
		No of diets exceeding ADI:		---					
Highest calculated TMDI values in % of	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)	
20.1	DE child	12.1	Apples	3.8	Oranges	1.3	Table grapes	19.8	
16.1	WHO Cluster diet B	9.6	Olives for oil production	1.8	Wine grapes	1.0	Apples	6.1	
12.2	NL child	6.3	Apples	3.1	Oranges	0.8	Table grapes	12.2	
8.3	ES child	3.7	Olives for oil production	2.2	Oranges	1.1	Apples	4.4	
7.4	IE adult	1.3	Wine grapes	1.0	Oranges	0.8	Apples	7.1	
6.9	PT General population	2.5	Wine grapes	1.3	Olives for oil production	1.1	Apples	5.4	
6.7	FR all population	4.0	Wine grapes	1.0	Olives for oil production	0.5	Apples	5.7	
5.7	ES adult	2.1	Olives for oil production	1.3	Oranges	0.8	Apples	3.4	
5.6	FR toddler	2.6	Apples	2.0	Oranges	0.3	Mandarins	5.6	
5.1	WHO cluster diet E	1.6	Wine grapes	0.8	Apples	0.8	Olives for oil production	4.2	
4.6	UK Toddler	2.0	Oranges	1.7	Apples	0.3	Mandarins	4.6	
4.3	NL general	1.5	Oranges	1.2	Apples	0.6	Wine grapes	4.3	
4.1	FR infant	2.5	Apples	0.9	Oranges	0.3	Pears	4.1	
3.7	DK child	2.3	Apples	0.9	Pears	0.2	Table grapes	3.7	
3.5	UK Infant	1.6	Apples	0.3	Oranges	0.3	Pears	3.5	
3.3	WHO regional European diet	0.7	Apples	0.7	Olives for oil production	0.5	Oranges	2.4	
3.3	SE general population 90th percentile	1.1	Apples	0.7	Oranges	0.4	Mandarins	3.0	
3.1	PL general population	2.0	Apples	0.3	Table grapes	0.3	Pears	3.1	
2.9	WHO Cluster diet F	0.9	Oranges	0.7	Apples	0.6	Wine grapes	2.9	
2.8	DK adult	1.4	Wine grapes	0.8	Apples	0.2	Pears	2.8	
2.8	IT kids/toddler	0.9	Apples	0.5	Oranges	0.3	Peaches	2.7	
2.8	UK vegetarian	0.9	Oranges	0.8	Wine grapes	0.6	Apples	2.7	
2.5	IT adult	0.8	Apples	0.4	Peaches	0.4	Oranges	2.4	
2.4	UK Adult	1.1	Wine grapes	0.6	Oranges	0.4	Apples	2.4	
2.3	WHO cluster diet D	0.7	Apples	0.4	Wine grapes	0.2	Oranges	2.3	
2.2	LT adult	1.9	Apples	0.2	Pears	0.1	Oranges	2.2	
2.0	FI adult	1.0	Oranges	0.4	Apples	0.3	Wine grapes	1.9	

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI. A long-term intake of residues of AMITROLE is unlikely to present a public health concern.

B.7.13.2. Risk assessment for acute exposure through diet

When amitrole was first peer-reviewed (Review Report (6839/VI/97-final 22 march 2001)), the setting of an acute reference dose was not deemed relevant in view of the hazard profile of the substance. This argument was also considered by EFSA in its reasoned opinion (EFSA 2012).

Nevertheless, in context of AIR II and according to B.6.10.2, new considerations lead to propose an ARfD for amitrole. An ARfD should be set based on the lowest NOAEL observed in the short-term and teratology toxicity studies. The most relevant study appear to be the 28-day toxicity study performed in rat, where the NOAEL was 1.5 mg/kg bw/d, based on decreased body weight and food consumption and decreased T3 and T4 levels. Therefore the following ARfD can be derived, with a safety factor of 100:

$$\text{ARfD} = \text{NOAEL} / \text{SF} = 1.5 \text{ mg/kg bw/d} / 100 = \mathbf{0.015 \text{ mg/kg bw}}$$

Consequently risk assessment for acute exposure through diet should be done. Since a no residue situation was observed for all intended crops, the lowest LOQ of 0.01mg/kg was considered.

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Table 7.13.2.-1 : EFSA model (PRIMo) for acute risk assessment - rev. 2 for amitrole

Acute risk assessment /children						Acute risk assessment / adults / general population						
The acute risk assessment is based on the ARfD.												
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.												
In the IESTI 1 calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.												
In the IESTI 2 calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variability factor of 3.												
Threshold MRL is the calculated residue level which would lead to an exposure equivalent to 100 % of the ARfD.												
Unprocessed commodities	No of commodities for which ARfD/ADI is exceeded (IESTI 1):			---			No of commodities for which ARfD/ADI is exceeded (IESTI 2):			---		
	IESTI 1		*)	**) ***)	IESTI 2		*)	**) ***)	IESTI 1		*)	**) ***)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	8.8	Oranges	0.01/-	6.4	Oranges	0.01/-	2.1	Table grapes	0.01/-	2.1	Table grapes	0.01/-
	6.5	Apples	0.01/-	5.9	Grapefruit	0.01/-	1.7	Oranges	0.01/-	1.6	Wine grapes	0.01/-
	6.1	Pears	0.01/-	4.8	Apples	0.01/-	1.6	Wine grapes	0.01/-	1.4	Oranges	0.01/-
	5.9	Grapefruit	0.01/-	4.4	Pears	0.01/-	1.5	Apples	0.01/-	1.2	Apples	0.01/-
	4.4	Table grapes	0.01/-	4.4	Table grapes	0.01/-	1.4	Pears	0.01/-	1.1	Pears	0.01/-
No of critical MRLs (IESTI 1)						No of critical MRLs (IESTI 2)						
---						---						
Processed commodities	No of commodities for which ARfD/ADI is exceeded:			---			No of commodities for which ARfD/ADI is exceeded:			---		
			*)			*)			*)			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)
	3.4	Apple juice	0.01/-	0.7	Orange juice	0.01/-	0.4	Apple juice	0.01/-	0.3	Wine	0.01/-
	3.3	Orange juice	0.01/-	0.4	Apple juice	0.01/-	0.3	Wine	0.01/-	0.1	Peach preserved	0.01/-
	2.2	Grape juice	0.01/-	0.3	Wine	0.01/-	0.1	Peach preserved	0.01/-	0.1	Quince jelly	0.01/-
	1.2	Peach juice	0.01/-									
	1.2	Pear juice	0.01/-									
*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported.												
**) pTMRL: provisional temporary MRL												
***) pTMRL: provisional temporary MRL for unprocessed commodity												
Conclusion:												
For AMITROLE IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.												
No exceedance of the ARfD/ADI was identified for any unprocessed commodity.												
For processed commodities, no exceedance of the ARfD/ADI was identified.												

B.7.14. SUMMARY AND EVALUATION OF RESIDUE BEHAVIOUR (ANNEX IIA 6.10, ANNEX IIIA 8.9)

The toxicological profile of amitrole was evaluated at EU level for the first inclusion. Since this time, new considerations are proposed in the context of AIR II to define an ARfD. An ARfD should be set based on the lowest NOAEL observed in the short-term and teratology toxicity studies. With a safety factor of 100, an ARfD is proposed at 0.015 mg/kg bw.

Primary crop metabolism of amitrole was investigated in apple to define residue for enforcement and risk assessment in crops under consideration. New metabolism studies are not deemed necessary with regard to intended uses on orchards, grapes and considering the same mode of application on the soil in early season. Additionally a different consideration for the use on olive is not deemed necessary since the $\log P_{ow}$ of amitrole is very low and in all likelihood with poor affinity with high lipid content matrix.

Since the first inclusion, new trials were provided on apple, grape and olive. Previously, stability was sufficiently demonstrated in high water, high acid and high lipid content matrices under frozen condition. Trials were mainly realized in the presence of the synergist ammonium thiocyanate and demonstrated that residue levels are below the LOQ in fruits. This confirms that no MRL exceedance will result from the intended uses. This assessment goes on the same way than the EFSA reasoned opinion about the review of the existing maximum residue levels (MRLs) for amitrole according to Article 12 of the Regulation (EC) No 396/2005.

Therefore, regarding to the no residue situation in those crops and the possibility for extrapolation given in guideline appendix D rev.9, sufficient number of residue trials are available to support all intended GAPs for amitrole on orchards, grapes and olives.

In addition, new residue results were also provided on metabolites triazolylalanine and aminotriazolylalanine in olives and grapes where a no residue situation was also shown. Consequently, the need to include these metabolites in the residue definition is not considered necessary with regard to the intended uses. The residue definition in plants for monitoring and risk assessment remains the parent amitrole.

As quantifiable residues of amitrole are not expected in the treated crops, there is no need to investigate the effect of industrial and/or household processing. Although not required, one study investigating the magnitude of residues in grapes, juice, must and wine (white and red) was provided. Nevertheless no robust processing factors could be derived since residues were below the LOQ in both raw and processed commodities.

Orchards, grapes and olives are not expected to be grown in rotation. Further investigation of residues in rotational crops is therefore not required with regard to the intended uses. Nevertheless further information would be required in future if additional uses are intended and rotated and when, at EU level, a full assessment report about triazoles derivatives metabolites will be available.

Since the first inclusion, studies on the nature or magnitude of residues in commodities of animal origin are not available nor required since livestock is not expected to be exposed to amitrole residues. Residue definitions and MRLs for livestock products are consequently not required.

Chronic and acute consumer exposures resulting from the uses proposed in the framework of this application were calculated. Based on EFSA PRIMo (rev2), acute and chronic exposures were considered as acceptable for all groups of consumers as no ARfD nor ADI exceedances are expected.

B.7.15. REFERENCES RELIED ON**B.7.15.1. New studies since first inclusion**

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
KIIA 6.1.1/01	Clemson, A.D.	1997	Amitrole: determination of freezer storage stability of amitrole residues in grapes Generated by: Huntington LifeSciences Document No: 95/CPF018/1407 GLP / GEP : Yes un-published	Y	NUFARM
KIIA 6.1.1/02	Biever, R.C.	2000	Amitrole – Stability in Apples Stored Under Frozen Conditions Generated by: Springborn Laboratories, Inc. Wareham, Massachusetts. Document No: 12983.0896.6128.395 95-08-6065, GLP / GEP : Yes un-published	Y	NUFARM
KIIA 6.1.1/03	Amic, S.	2011	Determination of Storage Stability of Amitrole in Grapes and Olives under Freezer Storage Conditions Generated by: EUROFINS ADME BIOANALYSIS Document No: S10-03264 GLP / GEP : Yes un-published	Y	NUFARM
KIIA 6.3.1/01	Wilson A.J.	1999	Generation of grape, must, wine and juice samples for determination of the magnitude of the residues of amitrole. Generated by : Viti R&D for the processing phase and Huntignton Lifesciences for the analytical phase Document No: CPF024/982343 GLP/GEP : yes un-published	Y	NUFARM
KIIA 6.3.1/02	Amic, S.	2012	Amitrole: residue study on grapes in France in 2011. Generated by: Eurofins ADME BIOANALYSE Document No: S11-01150 GLP / GEP : yes un-published	Y	NUFARM
KIIA 6.3.1/03	Provot, G.	1997	Determination de residus d'aminotriazole et de terbutylazine dans les echantillons de raisin traités avec le produit expérimental CA1397. Generated by: Centre International de Toxicologie (C.I.T) Document No: 11205 ADR – CFP404 GLP / GEP Yes un-published	Y	NUFARM

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
KIIA 6.3.2/01	Biever, R.C	1996	Amitrole: the accumulation and degradation of amitrole in apples. Generated by: Springborn Laboratories Inc. Document No: 95-08-6065/12983-0594-6107-395 GLP / GEP : Yes un-published	Y	NUFARM
KIIA 6.3.3/01	Amic, S.	2011	Amitrole – Residue Study on Olives in Spain in 2010 Generated by: Eurofins Document No: S10-03088 GLP / GEP : yes un-published	Y	NUFARM
KIIA 6.3.3/02	Amic, S.	2011	Amitrole – residue exploratory trial on processing olives in Spain in 2010. Generated by: Eurofins Document No: S10-035411 GLP / GEP : Yes un-published	Y	NUFARM
KIIA 6.3.3/03	Anonymous.	2000a	Análisis de residuos de 3-amino-1,2,4-triazole en aceitunas tratadas con ETIZOL TL por HPLC-fluorescencia. Generated by: Laboratory Agro-alimentari, Cabrils (E) Document No: report n° 401991S/1N-401992S/1N- 401993S/1N- 401995S/2N-401001B/N- 401001C/N-401001S/1N GLP/GEP : No un-published	Y	NUFARM
KIIA 6.3.3/04	Anonymous	2000b	Análisis de residuos de 3-amino-1,2,4-triazole en aceitunas tratadas con MAXATA TL por HPLC-fluorescencia. Generated by: Laboratory Agro-alimentari, Cabrils (E) Document No: report n° 401991S/2N- 401992S/2N-401993S/2N- 401995S/3N- 401002B/N-401002C/N-401002U/ GLP/GEP : No un-published	Y	NUFARM
KIIA 6.3.3/05	Schanne C.	1998	[¹⁴ C]-Amitrole : Isolation and characterisation of an Unknown Major Radioactive Fraction in Olives Generated by: Springborn Laboratories (Europe) AG, Health and Environmental Sciences, Seestrasse 21, CH-9326 Horn, Switzerland Document No: Springborn Rport # 97-044-1002 – Sprinborn Study # 1002.015.756 Source : CFPI AGRO GLP / GEP : Yes un-published	Y	NUFARM

B.7.15.2. References and scientific publications

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