UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

November 6, 2003

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Naphthaleneacetic Acid, Salts, Ester and Acetamide (056001, 056002, 056003, 056004, 056007, and 056008); Various Naphthaleneacetic Acid Studies/DER's (See List Below). DP Barcodes # (See List Below). Case 0379. MRID Nos.(See List Below).

FROM: Gary Otakie, Chemist
Reregistration Branch 4
Health Effects Division (7509C)

Gary Otakie

THROUGH: Susan V. Hummel, Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

Susan V. Hummel

TO: Mark Howard, Chemical Review Manager
Reregistration Branch 3
Special Review & Reregistration Division (7506C)

Goat Metabolism; DP Barcode D217162; MRID No. 43692301.
Storage Stability in Olives; DP Barcode D293169; MRID No. 44835301.
Olive Metabolism; DP Barcode D232643; MIRD No. 44190501.
Apple and Olive Processing; DP Barcode D294864; MRID Nos. 44586501 and 44555401.
Storage Stability in Apples and Pears; DP Barcode D295126; MRID Nos. 4466021 and 44660202.
Olive Field Trials; DP Barcode D295127; MRID No. 44555402.
Independent Laboratory Validation of Methods (ILV's) for Apples and Olives; DP Barcode D294853; MRID Nos. 44586502 and 44555403.

Attached are DER's of residue chemistry data submitted by AMVAC Corporation in response to data requirements in the Registration Standard. This information was reviewed by Dynamac Corporation under supervision of HED. The data assessment has undergone secondary review in the branch and has been revised to reflect division policies.

The adequacy of the data submitted to support the reregistration of NAA, its salts, ester, and acetamide will be evaluated in the upcoming RED.

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D217162, D232643, D294864, DD295126, D295127, and D294853
PC Codes: 056001, 056002, 056003, 056004, 056007, and 056008.

NAA/PC Code 056002/Amvac Chemical Corporation
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Olive

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03

STUDY REPORT:

44835301 Hathaway, M. (1999) Frozen Storage Stability of Naphthaleneacetic Acid in Olives: Lab Project Number: 4-97-6: AA970304. Unpublished study prepared by Southern Testing and Research Laboratories, Inc (Wilson, NC) for Amvac Chemical Corporation (Los Angeles, CA). 66 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted an olive storage stability study with NAA (1-naphthaleneacetic acid). Untreated samples of homogenized olives were fortified with NAA at 1.0 ppm, and the fortified samples were then stored at -16 C for up to 365 days (12 months). At designated time intervals, samples of olives were analyzed for residues of NAA using HPLC/UV Method NAA-AM-002 which is adequate for the quantitation of NAA residues in olives based on concurrent method recoveries. The data indicate that residues of NAA are relatively stable under frozen storage conditions in olives for up to 365 days.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. Test Compound Nomenclature

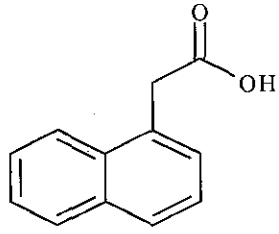
Compound	
Common name	NAA
Company experimental name	None
IUPAC name	1-Naphthaleneacetic acid
CAS name	
CAS #	86-87-3
End-use product/EP	Refer to the Residue Chemistry Chapter of the NAA RED

TABLE A.2. Physicochemical Properties of the Technical Grade NAA

Parameter	Value	Reference
Melting point/range	130 C	PC Chapter of the NAA RED
pH	3.45 (1% slurry; unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Density	3.75 lb/gal (unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Water solubility	420 mg/L	PC Chapter of the NAA RED
Solvent solubility	freely soluble in acetone, ether, and chloroform.	PC Chapter of the NAA RED
Vapor pressure	0.3 mm Hg at 26 C	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant, pK_a	4.3 (unspecified temperature)	RCB No. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient, $Log(K_{ow})$	Not applicable; TGAI is very polar	RCB No. 3468 and 3469, 6/3/88, F. Suhre
UV/visible absorption spectrum	Not available	

B. EXPERIMENTAL DESIGN

B.1. Sample Preparation

Samples of untreated homogenized olives were placed into Ziplock plastic bags and fortified with NAA at ~1 ppm. The fortified samples were then stored frozen at -16 C. The NAA fortification standard was prepared in acetonitrile (ACN) and water. Samples of unfortified olives (control samples) were also stored frozen.

B.2. Analytical Methodology

Samples of fortified and unfortified olives were analyzed for residues of NAA using Method NAA-AM-002 following 118, 193, 273, and 365 days of frozen storage. Fresh fortification samples were analyzed for concurrent method recoveries.

Briefly, homogenized samples of olives were blended with water, filtered, and boiled with 50% sodium hydroxide. The extract was filtered, washed with dichloromethane (DCM), and acidified to <pH 2 with concentrated hydrochloric acid. The acidified extract was then partitioned (2x) with DCM, and the resulting organic phase was dried over sodium sulfate and concentrated. An aliquot of the concentrate was purified through a silica gel solid-phase extraction (SPE-Si) cartridge; residues were eluted with DCM:acetic acid (99:1, v:v). The eluate was concentrated and redissolved in mobile phase (ACN:0.025 M KH_2PO_4, pH 5.0; 40:60, v:v) for HPLC/UV analysis using a SAX column. The validated limit of quantitation (LOQ) was 0.01 ppm for olives.

C. RESULTS AND DISCUSSION

The concurrent method validation data included in the study indicate that the HPLC/UV method (NAA-AM-002) is adequate for the determination of residues of NAA in/on olives. Apparent residues were nondetectable in all control (unfortified) olive samples (n = 5). The results indicate that residues of NAA appear to be stable in olives stored frozen for up to 365 days (12 months).

TABLE C.1. Summary of Concurrent Recoveries of NAA from Olives.

Matrix	Analyte	Spike level (ppm)	Storage Interval (days)	Sample size (n)	Recoveries (%)	Mean ± std dev
Olives	NAA	~1.0 [1]	0	3	106, 110, 121	112 ± 7.8
			118 [2]	2	74.4, 79.6	77.0 ± 3.7
			193	2	78.6, 96.1	87.4 ± 12.3
			273	2	81.1, 81.5	81.3 ± 0.3
			365	2	71.2, 84.5	77.9 ± 9.4

[1] Fortification levels ranged 1.00-1.04 ppm; however, actual fortification levels (used for recovery calculations) were not presented for individual samples.

[2] The original 3 month fresh fortification recoveries were unacceptable (<70%); new fresh fortification samples were re-extracted 27 days later and acceptable recoveries (reported above) were obtained.

FIGURE C.1. Graph of Residue Stability in Olives.

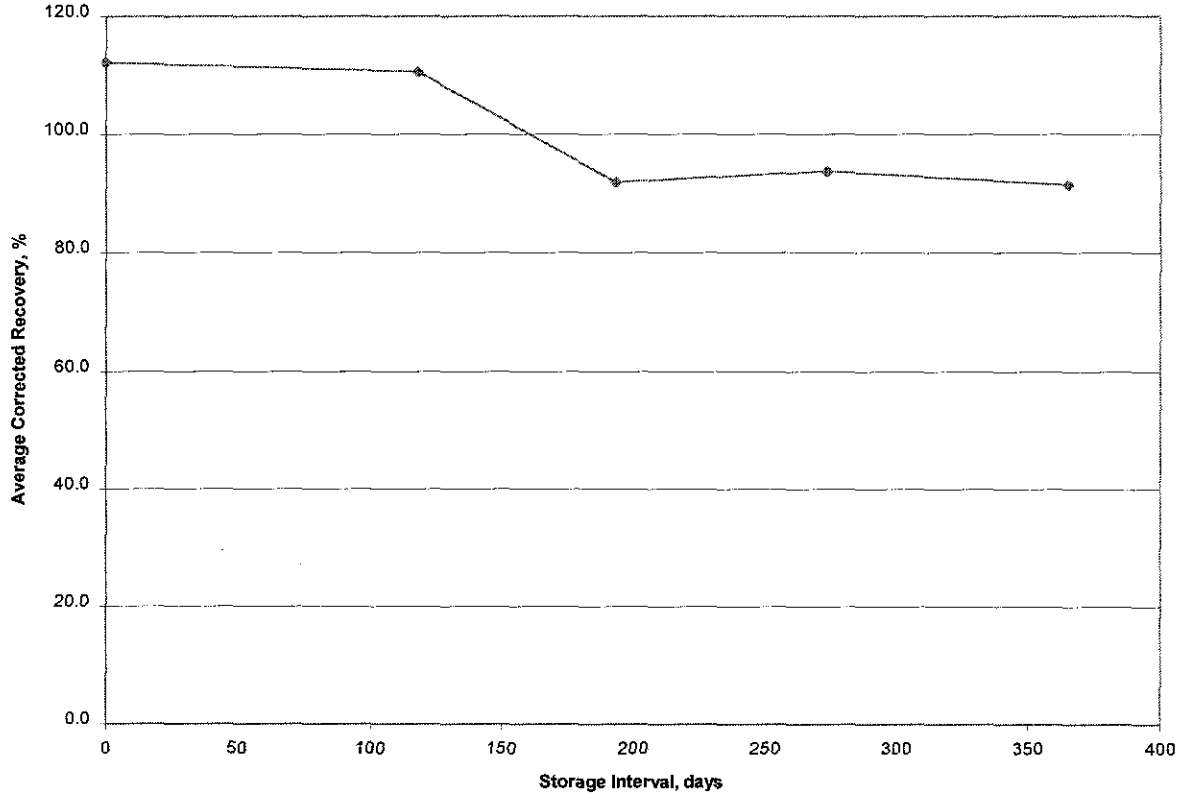


TABLE C.2. Stability of NAA Residues in Olives Following Storage at -16 °C.				
Commodity	Spike level (ppm)	Storage interval (days)	Recovered residues (ppm) [1]	Corrected % recovery [2]
Olives	~1.0	0	1.082, 1.135, 1.217 [3]	--
		118	0.855, 0.871, 0.915	107, 110, 115
		193	0.79, 0.825, 0.855	88.4, 91.8, 96.2
		273	0.733, 0.781, 0.798	90.0, 95.4, 96.1
		365	0.639, 0.764, 0.79	81.6, 94.5, 98.2

[1] Recovery values differ slightly when calculated from the ppm values found, because the registrant used actual fortification levels ranging 1.00-1.04 ppm in calculating recoveries; fortification levels were not reported for individual samples.
[2] Corrected for average concurrent-recoveries.
[3] Fresh fortification samples, refer to Table C.1. for percent recoveries.

D. CONCLUSION

The submitted storage stability study demonstrates that residues of NAA are relatively stable under frozen storage conditions in olives for up to 365 days.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (10/02/03); Susan V. Hummel (10/02/03)
Petition Number(s): None
DP Barcode(s): D293169
PC Codes: 056002

Template Version April 2003

NAA and NAA-OEt/PC Codes 056002 and 056008/Amvac Chemical Corporation
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Olive

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03

STUDY REPORT:

44190501 Obrist, J.; Crabtree, K.; Larson, J. (1996) NAA: Olive Metabolism Study Using Naphthaleneacetic Acid and Naphthaleneacetic Acid, Ethyl Ester: Final Report: Lab Project Number: 95458. Unpublished study conducted by ABC Laboratories (Madera, CA) for Amvac Chemical Corporation (Los Angeles, CA). 107 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted an olive metabolism study. A single olive tree was sequentially treated with radiolabeled naphthaleneacetic acid ethyl ester (NAA-OEt) and naphthaleneacetic acid (NAA). Each test substance was radiolabeled on the C-1 position of the naphthalene ring. The NAA-OEt test substance was applied as a 1% ai solution covering about 10% of the tree's new growth tips using a small brush. The NAA test substance was applied using a backpack sprayer as a spray solution at a concentration of 145 ppm ai 62 days following treatment of the same tree with NAA-OEt. Samples of treated olives were harvested at maturity, 118 days following the second application. The harvested olives were rinsed with acetonitrile, pitted, and the olive meat homogenized, extracted with acetonitrile and partitioned into organic and aqueous fractions. Conjugates were released using base hydrolysis. Radioactivity was analyzed by liquid scintillation spectrometry (LSC) with identification by reverse phase HPLC/UV and flow through a radioactivity detector. The total radioactive residues (TRR) were 0.018 ppm in/on whole olive. The major residues found were NAA (8.4%) and conjugates of NAA (55.4%).

There was no NAA-OEt detected in any fraction which was expected due to application of the test substance early in the growing season (without fruit). The remaining extractable radioactivity (55.4% TRR) was characterized to be comprised of five unknowns each present at ≤0.005 ppm. These unknowns were converted to NAA following base hydrolysis of the extracts, and therefore, were characterized as conjugates of NAA. Re-analysis of the organic and aqueous fractions of the extracts at the conclusion of the study demonstrated that residues were stable for the duration of the study.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the olive metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. Test Compound Nomenclature

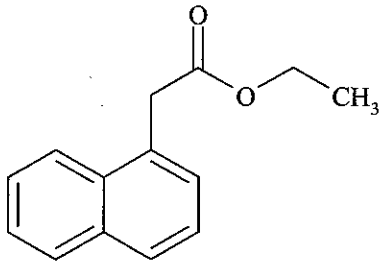
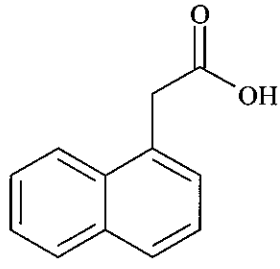
Compound		
Common name	NAA, ethyl ester	NAA
Company experimental name	None	None
IUPAC name	Ethyl 1-naphthaleneacetate	1-Naphthaleneacetic acid
CAS name	1-Naphthaleneacetic acid, ethyl ester	
CAS #	2122-70-5	86-87-3
End-use product/EP	Refer to the Residue Chemistry Chapter of the NAA RED	

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound			
Parameter	NAA-OEt	NAA	
	Value	Value	Reference
Boiling point/range	>150 C	130 C	PC Chapter of the NAA RED
pH	Not available	3.45 (1% slurry; unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Density	1.11 at 20 C	3.75 lb/gal (unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Water solubility	Not available	420 mg/L	PC Chapter of the NAA RED
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	freely soluble in acetone, ether, and chloroform.	PC Chapter of the NAA RED
Vapor pressure	Not available	0.3 mm Hg at 26 C	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant, pK_a	Not available	4.3 (unspecified temperature)	RCB No. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient, $Log(K_{OW})$	Not available	Not applicable; TGAI is very polar	RCB No. 3468 and 3469, 6/3/88, F. Suhre
UV/visible absorption spectrum	Not available	Not available	

B. EXPERIMENTAL DESIGN

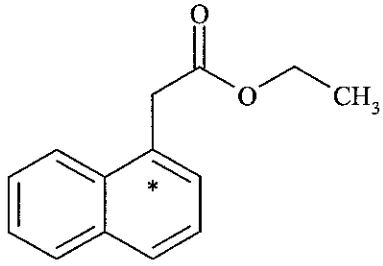
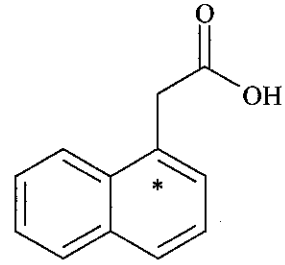
B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information				
Testing Environment	Soil characteristics			
	Type	% OM	pH	CEC (meq/100 g)
Fenced outdoor plots, each containing a single olive tree, at a commercial olive orchard in Madera, CA.	Sandy loam	0.3-0.6	6.9-7.4	5.1-6.9

Daily minimum and maximum temperatures and relative humidity, and precipitation, ground temperatures, and wind speed were reported throughout the study period. Drip irrigation was also provided as needed for normal tree growth. Although historical weather data were not provided, the registrant reported that the recorded climatic conditions were typical for the area.

TABLE B.1.2. Crop Information					
Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Olive/ Miscellaneous Crop	Sevillano	#1 - Early spring on new growth tips #2 - Sixty-two days after first treatment; 12-18 days after full bloom	Mature; 118 days after the last (second) treatment	Olives	Hand-picked

Test Materials

TABLE B.2.1. Test Material Characteristics		
Chemical structure		
Radiolabel position	1-C position of the naphthalene ring	1-C position of the naphthalene ring
Lot No.	CSL-94-538-49-28	CSL-94-538-51-20
Purity	≥98% (TLC)	≥98% (TLC)
Specific activity	43 mCi/mmol	43 mCi/mmol
Code	[^{14}C]NAA-OEt	[^{14}C]NAA

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information	
Chemical name	[^{14}C]naphthaleneacetic acid, ethyl ester and [^{14}C]naphthaleneacetic acid
Application methods and rates	The NAA-OEt test substance was applied as a 1% ai solution covering about 10% of the tree's new growth tips using a small brush. The NAA test substance was applied using a backpack sprayer as a spray solution at a concentration of 145 ppm ai 62 days following treatment of the same tree with NAA-OEt.
Total Number of Applications Made to the Olive Test Tree	2
Timing of applications	The first application, [^{14}C]NAA-OEt, was applied early spring. The second application, [^{14}C]NAA, was applied 62 days later, 12-18 days after full-bloom.
PHI	118 days

A second olive tree was not treated for control samples.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation

Whole olives were initially rinsed with acetonitrile (ACN). The rinsed fruit were then pitted by hand using a paring knife, and the flesh was weighed and homogenized with dry ice; the pits

were not analyzed. Duplicate samples of homogenized olive flesh were then extracted (2x) with ACN and filtered under vacuum. The filtrates were concentrated and partitioned (3x) with hexane. The hexane phases were combined, but contained no radioactivity and were not further analyzed. The remaining ACN:water phase was diluted with water and partitioned (2x) with dichloromethane (DCM). The aqueous phase was adjusted to pH 1-2 and partitioned (2x) again with DCM. All of the organic phases were combined. The aqueous phase and combined organic phases from the duplicate samples of olives extracted were combined. The organosoluble residues were concentrated for HPLC analysis, and the aqueous-soluble residues were adjusted to pH 5-6, lyophilized, and redissolved in water:methanol (7.5:0.5, v:v) for HPLC analysis.

Subsamples of the aqueous and organic phases were separately subjected to base hydrolysis (refluxing in 10% NaOH for 3.5 hours) for further characterization. The resulting hydrolysates were partitioned with DCM, adjusted to pH 1-2, and then repartitioned with DCM. The acidified DCM fractions were concentrated for HPLC analysis.

B.4.2. Analytical Methodology

Triplicate subsamples of flesh were combusted and radioassayed by liquid scintillation spectrometry (LSC), and the rinsate was radioassayed directly by LSC. The limit of detection (LOD) for the radioassay was 0.001 ppm. Total radioactive residues were calculated by the summation of the radioactivity in the meat and rinsate. The radioactivity in all extracts were determined by LSC, and nonextractable radioactivity was determined by combustion/LSC.

Samples of the rinsate, organic and aqueous phases following DCM partitioning, and acidified DCM fractions of the base hydrolysate were analyzed by reverse-phase HPLC. The RP-HPLC system was equipped with an ODS column, UV (280 nm) and flow-through radioactivity detector. A gradient mobile phase of water and ACN, each with 1% acetic acid, was used. Residues were identified by co-chromatography with unlabeled reference standards of NAA, NAA-OEt, and NAD (1-naphthylacetamide).

The identity of NAA in the organic phase of the olive extract was confirmed using 2D-TLC. TLC analysis was conducted on silica gel 60 F_{254} plates with a solvent system of toluene:acetone:acetic acid (75:25:1, v:v:v) in the first dimension and hexane:ethyl acetate:acetic acid (60:30:1, v:v:v) in the second dimension. Nonlabeled reference standards were co-chromatographed with the sample. Radioactivity was detected by radioanalytical imaging and nonradioactive zones were visualized by UV light.

C. RESULTS AND DISCUSSION

TRR in olives are reported in Table C.2.1. TRR were 0.018 ppm in/on whole olives harvested 118 days following the treatment schedule described. Based on the weight of the olive flesh, the total radioactive residues (TRR) was 0.003 ppm in the acetonitrile rinsate and 0.015 ppm in the

flesh, for a total of 0.018 ppm in/on the whole fruit. Following the acetonitrile rinse, an additional 63.1% of whole fruit TRR was extracted with acetonitrile; the nonextractable residues were 19.2% (0.003 ppm) of the whole fruit TRR. The radioactivity in the acetonitrile extract was partitioned into organic and aqueous fractions. The distribution of the radioactivity in olives is presented in Table C.2.2. The characterization and identification of residues are summarized in Table C.2.3. The majority of the TRR was removed as surface residues (16% TRR) or extracted (84% TRR) from olives using acetonitrile. Extractable residues were partitioned into organic- and aqueous-soluble fractions. Nonextractable residues were 19% of the TRR (0.003 ppm) in olives following solvent extraction.

The free form of the parent, NAA, was the only component identified in olives at 8.4% TRR. The remaining extractable radioactivity was characterized as five unknowns accounting for 55.4% TRR (0.010 ppm); each unknown was present at ≤0.005 ppm. Residues were characterized/identified by HPLC analysis, and NAA was confirmed by TLC analysis. The unknowns were converted to free NAA with base hydrolysis of the organic and aqueous phases of the extract, and therefore characterized as conjugates of NAA.

C.1. Storage Stability

The initial extraction and HPLC analyses of the extracts occurred within 181 days (~6 months) of harvest. The registrant reported that essentially identical results were obtained for the initial and later analyses which demonstrated that residues in the aqueous and organic fractions were stable for the duration of the study.

TABLE C.1. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (C)	Actual Study Duration	Limit of Demonstrated Storage Stability
Olives	-18	181 days (6 months)	Re-analysis of the organic and aqueous extracts 184 days (6.1 months) after the initial analyses.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Olives.

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm)
Olive, rinsate	2	118	0.003
Olive, flesh			0.015
Total			0.018

TABLE C.2.2. Distribution of the Parent and the Metabolites in Olives Following Sequential Applications of [^{14}C]NAA-OEt and [^{14}C]NAA.

Metabolite Fraction	Whole Olives [1] (TRR = 0.018 ppm)	
	%TRR	ppm
ACN Rinsate	16.1	0.003
NAA	1.5	<0.001
Unknown A	0.6	<0.001
Unknown B1	0.5	<0.001
Unknown B2	11.4	0.002
Unknown C	0.8	<0.001
Uncharacterized	1.3	<0.001
Olive Flesh	83.9	0.015
ACN extract	63.1	0.012
Hexane	None detected	
DCM	25.2	0.005
NAA	5.4	0.001
Unknown A	0.7	<0.001
Unknown B1	10.5	0.002
Unknown B2	3.7	<0.001
Unknown C	3.0	<0.001
Unknown D	0.8	<0.001
Void Volume	0.3	<0.001
Uncharacterized	0.8	<0.001
Aqueous	28.8	0.006
NAA	1.5	<0.001
Unknown A	5.0	0.001
Unknown B1	4.1	<0.001
Unknown B2	13.5	0.003
Unknown C	0.8	<0.001
Void Volume	0.5	<0.001
Uncharacterized	3.4	<0.001
Total extractable	70.1	0.013
Total identified	8.4	0.002
Total unidentified	61.7	0.011
Total bound residues (PES)	19.2	0.003
% Accountability	89.3	

[1] Values are reported as presented by the registrant.

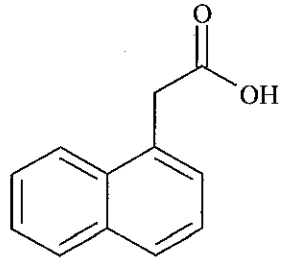
TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Olives Following Sequential Applications of [^{14}C]NAA-OEt and [^{14}C]NAA.

Compound	Whole Olives (TRR = 0.018 ppm)	
	%TRR	ppm
Identified:		
NAA	8.4	0.002
Characterized as conjugates of NAA following base hydrolysis of extracts:		
Unknown A	6.3	0.001
Unknown B1	15.1	0.003
Unknown B2	28.6	0.005
Unknown C	4.6	<0.001
Unknown D	0.8	<0.001
Uncharacterized extractable residues (includes void volume)	6.3	0.001
Total identified	8.4	0.002
Total characterized	55.4	0.010
Total extractable	70.1	0.013
Total bound	19.2	0.003

C.3. Proposed Metabolic Profile

No metabolic pathway was presented.

TABLE C.3.1. Identification of Compounds from Olive Metabolism Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
NAA	1-Naphthaleneacetic acid	

D. CONCLUSION

The total radioactive residues in/on mature whole olive fruit were 0.018 following sequential applications of [^{14}C]NAA-OEt and [^{14}C]NAA to the same tree at the respective rates described. NAA was the only residue identified and characterized in olives for a total of 63.8% of TRR (8.4% of TRR was free NAA and 55.4% was as conjugates). There was no NAA-OEt detected in any fraction which the registrant believes is not surprising due to the rapid degradation in plants and the fact that NAA-OEt was applied at the beginning of the growing season when the test olive tree did not bear fruits. The Agency concurs with this observation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (10/02/03); Susan V. Hummel (10/02/03)
Petition Number(s): None
DP Barcode(s): D232643
PC Codes: 056002 and 056008

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NAA/PC Code 056002/Amvac Chemical Corporation
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Nature of the Residues in Livestock - Goat

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03	Gary Otakie
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03	Susan V. Hummel

STUDY REPORT:

43692301 Krautter, G.; Downs, J.; Marsh, J. (1995) The Metabolism of [^{14}C]-1-Naphthylacetic Acid in the Lactating Goat: Lab Project Number: 870: 1673. Unpublished study conducted by PTRL East, Inc. (Richmond, KY) for Amvac Chemical Corporation (Los Angeles, CA) 95 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted a ruminant metabolism study with NAA. A single lactating goat was orally dosed once daily with [^{14}C]NAA at 10 ppm in the diet for three consecutive days. The test substance was radiolabeled on the C-1 position of the naphthalene ring. Milk was collected twice daily throughout the study, and tissues (muscle, fat, liver, and kidney) were collected at animal sacrifice.

Total radioactive residues (TRR) were <0.001-0.050 ppm in milk, <0.001 ppm in muscle, 0.003 ppm in fat, 0.005 ppm in kidney, and 0.009 ppm in liver. The study provided data which showed that the majority of the administered dose was excreted in urine (87.3%) and that low levels of radioactivity were excreted in the feces (1% of dose).

TRR levels in milk were consistently higher in samples collected 0-8 hours after each dosing period than those samples collected 8-24 hours following dosing. The 48-56 hour milk sample was fractionated, and the majority of the radioactivity (98.5% TRR) was determined in the skim milk fraction. Chromatographic analysis of the skim milk detected the glycine conjugate of NAA which accounted for virtually all of milk TRR.

The TRR in all goat tissue samples were each <0.01 ppm which is below the Agency trigger value for residue characterization and identification. Nonetheless, the registrant performed analytical work on liver. Sequential extraction of the liver sample with ACN:water, water, and hexane released 36.7% of TRR. The nonextractable residue in liver comprised ~63% of TRR and was not subjected to additional fractionation procedures because of low radioactivity level (0.006 ppm). The extractable liver residues were partitioned into organic and aqueous-soluble fractions. The majority of the extractable radioactivity was determined in the aqueous fraction which was subjected to acid hydrolysis to release conjugated metabolites. The principal residue

component identified in liver was the free and conjugated forms of NAA which jointly accounted for 19.7% of TRR (<0.002 ppm). An unknown was also characterized as a minor residue (<4% TRR, <0.001 ppm) in liver. NAA and its conjugates were quantitated by HPLC with flow-through radioactivity detection, and the NAA glycine conjugate in milk was confirmed by TLC with radioanalytical imaging with the glycine conjugate metabolite identified in goat urine. Samples of milk and liver were extracted, hydrolyzed, and analyzed within 5 months of collection; therefore, supporting storage stability data are not required.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the livestock metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

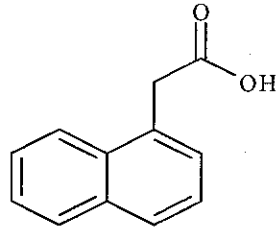
COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379 and the PC Codes are listed below.

TABLE A.1. Test Compound Nomenclature

Compound	
Common name	NAA
Company experimental name	None
IUPAC name	1-Naphthaleneacetic acid
CAS name	

CAS #	86-87-3
End-use product/EP	Refer to the Residue Chemistry Chapter of the NAA RED

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound (NAA)

Parameter	Value	Reference
Melting point/range	130 C	PC Chapter of the NAA RED
pH	3.45 (1% slurry; unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Density	3.75 lb/gal (unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Water solubility	420 mg/L	PC Chapter of the NAA RED
Solvent solubility	freely soluble in acetone, ether, and chloroform.	PC Chapter of the NAA RED
Vapor pressure	0.3 mm Hg at 26 C	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant, pK_a	4.3 (unspecified temperature)	RCB No. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient, $Log(K_{ow})$	Not applicable; TGAI is very polar	RCB No. 3468 and 3469, 6/3/88, F. Suhre
UV/visible absorption spectrum	Not available	

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Lactating goat (*Capra hircus*)	"cross-bred"	~3 years	27.5 (control) 43.0 (treated)	Good general health	Stainless steel metabolism cages maintained in the animal facility of PTRL East (Richmond, KY), at 13-26 C, 63-88% Rh, and 16/8 hours light/dark cycle.

TABLE B.1.2. Test Animal Dietary Regime

Diet	Water	Acclimation period	Predosing
Unrestricted access to a combination of hay and a commercial high protein dairy goat feed.	Fresh potable water *ad libitum*	7 days	None

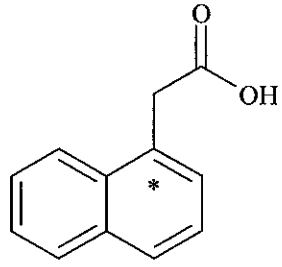
TABLE B.1.3. Test Animal Dosing Regime

Treatment Type	Level of administered dose (mg/day)	Food consumption (kg/day)	Residue intake in diet (ppm) [1]	Vehicle	Timing/Duration

Oral	16.5	1.650-2.195 (dose period)	10.0	Gelatin capsules using a balling gun	Once daily (after morning milking) for 3 consecutive days

[1] Based on the mean feed consumption (1.652 kg/day) during acclimation days 4-6.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics	
Chemical structure	
Radiolabel position	1-C position of the naphthalene rings
Lot No.	CSL-94-516-34-19
Purity	>99%
Specific activity	56.1 mCi/mmol
Code	[^{14}C]NAA

The [^{14}C]NAA was isotopically diluted with unlabeled NAA standard in acetone to a specific activity of 233,140 dpm/µg. The solvent was evaporated off after distribution into the gelatin capsules.

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Milk collected	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Milk was collected twice daily. For the treated goat: 909-1245 g/day collected during the acclimation period, and 834-889 g/day collected during the dosing period.	Urine and feces collected daily and cage wash collected at sacrifice	24 hours	Liver, kidneys, muscle and fat.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation

Milk was collected twice daily - in the morning prior to dosing and in the afternoon; morning and afternoon milk samples were kept separate. Triplicate aliquots of each milk sample were collected for TRR determination by LSC; the remaining milk samples were frozen. Tissue samples were cubed and frozen, and the frozen samples were homogenized in a food processor with dry ice for TRR determination by combustion/LSC. The limit of quantitation (LOQ) for combustion/LSC or LSC analysis was ≤0.001 ppm.

A subsample of milk (48-56 hour) was fractionated into cream, skim milk, and solids by centrifugation, and the skim milk fraction was analyzed by HPLC.

Only the liver sample was selected for residue characterization and identification because all other tissue samples contained TRR of ≤0.005 ppm. A subsample of liver was sequentially extracted with acetonitrile (ACN):water (2x; 1:1, v:v), water, and hexane. The ACN:water and water extracts were combined, concentrated, adjusted to pH 3 with 12 N HCl, and partitioned with ethyl acetate. The resulting organic and aqueous phases were reserved for HPLC analysis. A subsample of the aqueous phase was also subjected to acid hydrolysis (6 N HCl at ~100 C for 3 hours), and the hydrolysate was partitioned with ethyl acetate. The organic and aqueous phases were concentrated for HPLC analysis.

B.4.2. Analytical Methodology

The radioactivity in skim milk and the liver extracts was determined in triplicate by LSC, and nonextractable radioactivity was determined by combustion/LSC.

An aliquot of the fractionated skim milk, and the aqueous and/or organic phases of the liver extract before and after acid hydrolysis were analyzed by reverse-phase HPLC. The RP-HPLC system was equipped with a Zorbax SB-18 column, UV (280 nm) and flow-through radioactivity detector. A gradient mobile phase of water and ACN, each with 1% acetic acid, was used. Residues were identified by co-chromatography with the unlabeled reference standard of NAA.

HPLC analysis of an aliquot of skim milk fortified with goat urine, demonstrated that the unknown milk metabolite co-eluted with the conjugated urine metabolite. To confirm the milk metabolite was the same as in urine, a separate subsample of whole milk (48-56 hour) was acidified to pH 3 with 12 N HCl and partitioned with hexane. Following centrifugation, the organic layer was removed, and the milk was partitioned again. The resulting aqueous (skim milk) fraction and an aliquot of urine were analyzed by TLC; this confirmed that the milk metabolite was the glycine conjugate of NAA. TLC analysis was conducted on silica gel 60 F_{254} plates with a solvent system of chloroform:methanol:glacial acetic acid (24:8:1, v:v:v). Radioactivity was detected by radioanalytical imaging, and nonradioactive zones were visualized by UV light.

To demonstrate that the metabolite profile in milk did not change over the duration of the dosing period, the 0-8 hour milk sample was also analyzed by HPLC.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in goat milk and tissues from a single goat dosed orally with [1-^{14}C]NAA at 10 ppm in the diet for 3 consecutive days, are reported in Table C.2.1. TRR levels in milk were consistently higher in samples collected 0-8 hours after each dosing compared to samples collected 8-24 hours following dosing, demonstrating that residues were rapidly absorbed and distributed into milk. The majority of the administered dose was excreted in urine (87.3%); low levels of radioactivity were excreted in the feces (1% of dose).

The distribution of radioactivity in goat milk and liver is reported in Table C.2.2. Because the TRR in all tissue samples were <0.01 ppm, only the liver sample, with the highest TRR, was extracted for metabolite characterization. Sequential extraction of the liver sample with ACN:water, water, and hexane only released 36.7% TRR (<0.004 ppm). The majority of the TRR (~63% TRR) was nonextractable from goat liver; however, because of low radioactivity levels (0.006 ppm), no further work was conducted to characterize the nonextractable residues.

The 48-56 hour milk sample was fractionated and the majority of the radioactivity (98.5% TRR) was determined in the skim milk fraction. Radioactivity in solids following fractionation were not reported.

The characterization and identification of radioactive residues is summarized in Table C.2.3. Extractable liver residues were partitioned into organic and aqueous-soluble fractions. The majority of the extractable radioactivity was determined in the aqueous fraction which was subjected to acid hydrolysis to release conjugates of NAA. NAA (free and conjugated) was the only residue identified in goat liver, and accounted for 19.7% of the TRR (<0.002 ppm). An unknown was also characterized as a minor residue (<4% TRR, <0.001 ppm) in liver. The glycine conjugate of NAA was the only residue found in skim milk and accounted for 98.5% of the TRR (0.049 ppm) in milk. NAA and its conjugates were quantitated by HPLC, and the NAA glycine conjugate in milk was confirmed by TLC analysis with the glycine conjugate metabolite identified in goat urine.

HPLC chromatographic results were the same for the 0-8 hour and 48-56 hour milk sample, demonstrating that the only residue (glycine conjugate of NAA) in milk did not change over the duration of the dosing period.

C.1. Storage Stability

Sample of milk and goat tissues were stored frozen (-20 C) following collection. TRR determinations were made within 24 days of collection. Milk and liver samples were extracted and/or hydrolyzed and analyzed within 5 months of collection; therefore, no supporting storage stability data are required.

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (C)	Actual Storage Duration	Limit of Demonstrated Storage Stability
Goat, milk	-20	148 days (4.9 months)	None required
Goat, liver		89 days (2.9 months)	

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissues, and Excreta

Matrix	Collection Timing	TRR, ppm
Urine	Study duration	56.7% of Dose
Cage wash and solids	Sacrifice	30.6% of Dose
Feces	Study duration	1.0% of Dose
GI tract/bile	Sacrifice	0.4% of Dose
Total excreta	Study duration	88.7% of Dose
Muscle	Sacrifice	<0.001
Fat	Sacrifice	0.003
Kidney	Sacrifice	0.005
Liver	Sacrifice	0.009
Milk	0-8 hours	0.045
	8-24 hours	0.001
	24-32 hours	0.015
	32-48 hours	<0.001
	48-56 hours	0.050
	56 hours-sacrifice	0.001
Blood	Sacrifice	0.004
% of Administered Dose	Study duration-sacrifice	88.7% of Dose

TABLE C.2.2. Distribution of the Parent and the Metabolites in Goat Matrices when Dosed with [^{14}C]NAA[1].

Metabolite Fraction	Liver (TRR = 0.009 ppm)		Milk (48-56 hours) (TRR = 0.050 ppm)	
	%TRR	ppm	%TRR	ppm
ACN:water Extract	30.2	0.003		
Water Extract	3.4	<0.001		
-Pooled ACN:water and Water Extracts	33.6	0.003		
Organic Fraction	10.2	0.001		
NAA	5.1	<0.001		
NAA conjugate	1.5	<0.001		
Unknown M4	3.6	<0.001		
Aqueous Fraction	26.6	0.003		
Unknown M1	26.6	0.003		
Acid hydrolysate, organic phase	13.1	0.001		
NAA	13.1	0.001		
Acid hydrolysate, aqueous phase	15.8	0.002		
Hexane Extract	3.1	<0.001		
Skim milk			98.5	0.049
NAA glycine conjugate			98.5	0.049
Total extractable	39.9	0.004	98.5	0.049
Total identified	19.7	<0.002	98.5	0.049
Total unidentified	22.5	<0.003	--	--
Total bound residues (PES)	63.3	0.006	Not reported	
% Accountability	103.2		98.5	

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.

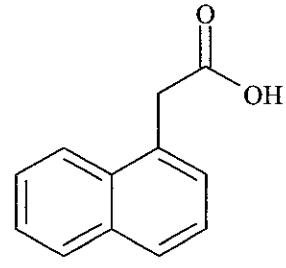
TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Dosing of Radiolabeled NAA at 10 ppm in the Diet.

Compound	Liver (TRR = 0.009 ppm)		Milk (48-56 hours) (TRR = 0.050 ppm)	
	% TRR	ppm	%TRR	ppm
NAA (free and conjugated forms)	19.7	<0.002	98.5	0.049
Unknown M4	3.6	<0.001	--	--
Hexane	3.1	<0.001	--	--
Acid hydrolysate, aqueous phase	15.8	0.002	--	--
Total identified	19.7	<0.002	98.5	0.049
Total characterized	22.5	<0.003	--	--
Total extractable	39.9	0.004	98.5	0.049
Total bound	63.3	0.006	Not reported	

C.3. Proposed Metabolic Profile

No metabolic pathway was presented; however, the registrant stated that NAA is readily absorbed through the gastrointestinal tract of the goat, and the absorbed residues are then quantitatively excreted in the urine as either free or conjugated forms of NAA. The lack of oxidative metabolites of NAA in urine or milk demonstrate that the primary metabolism route (oxidation) did not occur in goats.

TABLE C.3.1. Identification of Compounds from Goat Metabolism Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
NAA	1-Naphthaleneacetic acid	

D. CONCLUSION

The free or conjugated form of NAA was the only residue component identified in the milk and liver of a goat which was orally dosed with [^{14}C]NAA at 10 ppm in the diet for 3 consecutive days. Based on the results from this study, the registrant concluded that orally administered [^{14}C]NAA was readily absorbed through the gastrointestinal tract of the goat, and that absorbed residues were quantitatively excreted in the urine. Given the lack of evidence that oxidative metabolites of NAA were present in milk and liver, it appeared that primary (oxidative) metabolism played little or no role in the metabolism of NAA in the goat. HED concurs with the registrant's conclusions.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (10/02/03); Susan V. Hummel (10/02/03)
Petition Number(s): None
DP Barcode(s): D217162
PC Codes: 056002

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NAA/PC Code 056002/Amvac Chemical Corporation
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Independent Laboratory Validation - Apple and Olives

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03

FIRST STUDY REPORT (APPLES):

44586502 Hathaway, M. (1997) Independent Laboratory Validation of Analytical Method NAA-AM-001: Recommended Analytical Residue Method for 1-Naphthaleneacetic Acid, 1-Naphthaleneacetic, and/or 1-Naphthaleneacetic Acid Ethyl Ester, in Apple and Pear Matrices: Lab Project Number: AA960319: 4-96-2: NAA-AM-001. Unpublished study prepared by Southern Testing and Research labs., Inc. 72 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted the results of an independent laboratory validation (ILV) for a new analytical method, HPLC method NAA-AM-001 which quantitates residues of 1-naphthaleneacetic acid (NAA), 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester determined as the free acid (NAA) in fruit commodities. The ILV study was conducted by Southern Testing and Research Laboratories, Inc. (Wilson, NC).

Using Method NAA-AM-001, residues of 1-naphthaleneacetic acid, 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester are extracted from homogenized fruit samples by blending with water. Residues are converted to the parent 1-naphthaleneacetic acid by base hydrolysis followed by partitioning with dichloromethane. The aqueous phase is acidified to <pH 2 and partitioned again with dichloromethane. The resulting organic phase is dried over sodium sulfate, concentrated, and purified through a silica gel solid-phase extraction (SPE-Si) cartridge. The eluate is repeatedly concentrated with the addition of acetonitrile and finally redissolved in acetonitrile/water for analysis by HPLC using fluorescence detection. Residues are quantitated as the free acid (NAA), and a molecular weight conversion factor is applied to express residues in terms of the desired analyte (1-naphthalacetamide or 1-naphthaleneacetic acid ethyl ester). The method limit of quantitation (LOQ) is 0.01 ppm in/on apples and pears for each analyte in combination (expressed as NAA equivalents) or individually.

A successful ILV of Method NAA-AM-001 was completed. Acceptable recoveries were obtained from apples fortified with NAA at the method LOQ (0.01 ppm) and at 1.0 ppm. The subject method will be submitted to ACL/BEAD for additional regulatory method validation by Agency chemists.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the ILV data for HPLC method NAA-AM-001 are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. Test Compound Nomenclature

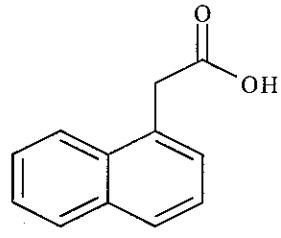
Compound	
Common name	NAA
Company experimental name	None
IUPAC name	1-Naphthaleneacetic acid
CAS name	
CAS #	86-87-3
End-use product/EP	Refer to the Residue Chemistry Chapter of the NAA RED

TABLE A.2. Physicochemical Properties of the Technical Grade NAA		
Parameter	Value	Reference
Melting point/range	130 C	PC Chapter of the NAA RED
pH	3.45 (1% slurry; unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Density	3.75 lb/gal (unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Water solubility	420 mg/L	PC Chapter of the NAA RED
Solvent solubility	freely soluble in acetone, ether, and chloroform.	PC Chapter of the NAA RED
Vapor pressure	0.3 mm Hg at 26 C	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant, pK_a	4.3 (unspecified temperature)	RCB No. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient, $Log(K_{OW})$	Not applicable; TGAI is very polar	RCB No. 3468 and 3469, 6/3/88, F. Suhre
UV/visible absorption spectrum	Not available	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Not applicable to this submission.

B.2. Enforcement Method

Amvac Chemical Corporation has submitted an ILV study for the HPLC method NAA-AM-001 entitled "Recommended Analytical Residue Method for 1-Naphthaleneacetic acid, 1-Naphthalacetamide, and 1-Naphthalene acetic acid, ethyl ester in Apple and Pear Matrices." The method parameters outlined in Table B.2.1 are from a method attachment to the ILV study.

Residue data for apples and pears, discussed in the 9/20/91 Residue Chemistry Update, were generated using an HPLC method in which NAA related residues are converted to the free acid which is then methylated using diazomethane and quantitated as the methyl ester. For safety reasons the Agency has recommended that the petitioner investigate using other procedures without diazomethane as the methylating reagent for an enforcement method. Method NAA-AM-001 eliminates the use of diazomethane, and residues are not methylated but are quantitated as the free acid (NAA).

B.2.1. Principle of the Method:

Residues of 1-naphthaleneacetic acid, 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester are extracted from homogenized samples of apple and pear matrices by blending with water. Residues are converted to the parent 1-naphthaleneacetic acid by base hydrolysis

followed by partitioning with dichloromethane (DCM). The aqueous phase is acidified to <pH 2 and partitioned again with dichloromethane. The resulting organic phase is dried over sodium sulfate, concentrated, and purified through a silica gel solid-phase extraction (SPE-Si) cartridge. The eluate is repeatedly concentrated with the addition of acetonitrile and finally redissolved in acetonitrile/water for analysis by HPLC using fluorescence detection. Residues are quantitated as the free acid (NAA), and a molecular weight conversion factor is applied to express residues in terms of the desired analyte (1-naphthalacetamide or 1-naphthaleneacetic acid, ethyl ester).

TABLE B.2.1. Summary Parameters for the HPLC Analytical Method Used for the Quantitation of NAA Residues in Apples and Pears.	
Method ID	NAA-AM-001
Analyte(s)	1-Naphthaleneacetic acid, 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester determined as the free acid (NAA)
Extraction solvent/technique	Homogenized samples of apple and pear matrices are blended with water. A 50% sodium hydroxide solution is added so that the resulting mixture contains at least 10% sodium hydroxide. An antifoam agent is added to prevent excessive bumping during hydrolysis at reflux for 3 hours. The extract is vacuum filtered through Whatman GF/A glass fiber filter media and partitioned with dichloromethane. The organic phase is discarded, and the aqueous phase is acidified to <pH 2 with concentrated hydrochloric acid. The acidic extract is then partitioned twice with dichloromethane. The resulting organic phase is collected through sodium sulfate and concentrated by rotary evaporation; the volume is adjusted with dichloromethane.
Cleanup strategies	An aliquot of the sample extract is again passed through sodium sulfate to ensure that all water is removed prior to cleanup through a SPE-Si column. Residues are eluted from the SPE-Si column with dichloromethane:acetic acid (99:1, v:v) and subjected to solvent exchange to be suitable for HPLC analysis. An aliquot of the SPE-Si eluate is concentrated under a stream of nitrogen gas at 35 °C, acetonitrile (ACN) is added, and the eluate concentrated again. The concentrated residues are then diluted with ACN, and water is added. The method notes that a light precipitate may form when the water is added. Residues in ACN:water (1:1, v:v) are filtered prior to analysis.
Instrument/Detector	HPLC utilizing a strong anion exchange (SAX) column with an isocratic mobile phase of acetonitrile:0.025 M KH_2PO_4 pH 5 buffer (12:88, v:v), and a fluorescence detector. The excitation wavelength is 220 nm, and the emission wavelength is 340 nm.
Standardization method	Linear regression from external calibration standards of 1-Naphthaleneacetic acid (NAA) prepared in ACN:water (1:1, v:v).
Stability of std solutions	No information or data concerning the stability of the standard solutions were included in the method. The method does specify that system suitability (linearity) be monitored daily.
Retention times	15.1-15.3 minute retention times for NAA based on representative chromatograms.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Not applicable to this submission.

C.2. Enforcement Method

The characteristics of the HPLC method NAA-AM-001 used in the ILV study are outlined in Table C.2.2. No additional method validation data were provided; however, based on the recoveries from the ILV study, the method adequately quantitates residues of NAA at the method LOQ (0.01 ppm) and 1.0 ppm in apples.

TABLE C.2.1. Recovery Results from Method Validation of [matrices] using the Enforcement Analytical Method.

Matrix	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Not applicable to this submission.			

TABLE C.2.2. Characteristics for the HPLC Analytical Method Used for the Quantitation of NAA Residues in Apples and Pears.

Analyte	1-Naphthaleneacetic acid (NAA)
Equipment ID	HPLC system utilizing a Thomson Advantage SAX column, or equivalent, with a fluorescence detector (Thermo Separation Products Fluor LC 304).
Limit of quantitation (LOQ)	0.01 ppm for apples and pears
Limit of detection (LOD)	Not provided
Accuracy/Precision	No method validation data, besides those from the ILV study, were provided to establish accuracy and precision.
Reliability of the Method/ [ILV]	An independent laboratory method validation (ILV) of HPLC method NAA-AM-001 was conducted using apple. The recovery values obtained with the second trial indicate that the HPLC method using fluorescence detection is reliable at the method LOQ (0.01 ppm) and 100x the LOQ for apples; see Section C.3 below.
Linearity	The instrument response was linear (coefficient of determination, r^2, was 0.9998) for NAA standards, prepared in ACN:water (1:1,v:v), in the range of 3-50 ng/mL.
Specificity	No matrix interference was observed in the control sample chromatograms (apple), and the analyte peak was well defined and symmetrical.

C.3. Independent Laboratory Validation

Amvac Chemical Corporation submitted an independent laboratory validation study (ILV; MRID 44586502) conducted by Southern Testing and Research Laboratories, Inc. (Wilson, NC) for the HPLC method NAA-AM-001. Untreated samples of homogenized apple, obtained locally, were fortified with 1-naphthaleneacetic acid (NAA) at 0.005 and 1.0 ppm in the first trial, and 0.01 (LOQ) and 1.0 ppm in the second trial. Fortified and unfortified (control) samples were analyzed using the HPLC method as described in Table B.2.1.

Adequate recoveries were obtained from apples fortified at the 1.0 ppm level with the first method trial; however, the first method trial failed for apples fortified at the lower level (0.005 ppm). Therefore, a second trial was conducted at the fortification levels 0.01 and 1.0 ppm. Adequate recoveries were obtained from apples at both fortification levels with the second

method trial. Recoveries of NAA from the ILV study are reported in Table C.3.1. No detectable residues of NAA (reported as 0.0000 ppm) were observed in any unfortified samples of apple.

The ILV laboratory noted that the method specifies use of a Thomson Advantage SAX HPLC column (or equivalent), which was unavailable at the start of the study. A DuPont Zorbax SAX HPLC column was initially used, as recommended by the method developers; however, the resulting chromatographic determinations produced analyte peaks that were significantly broader than the representative chromatograms included in the test method. Since resolution was a concern due to a noted interferent peak eluting immediately prior to the analyte peak in apple matrix, a Thomson Spherisorb SAX column was used to improve the peak shape. Finally, a Thomson Advantage SAX column was used which produced the best chromatography. Acceptable recoveries were obtained in the second trial using both the Thomson Spherisorb SAX and Zorbax SAX columns. In addition, the laboratory noted that some types of apples, specifically the Red Delicious variety, would not filter through the GF/A glass fiber filter specified in the test method. A Whatman #541 hardened ashless filter paper was found to be suitable as an alternative for these samples.

The performing laboratory reported that a skilled chemist should be able to extract and cleanup approximately 6 to 8 samples in a 2-day period. HPLC determinations may be automated and performed overnight following the second day of sample preparation.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the HPLC Analytical Method for the Determination of NAA in Apple.

Matrix	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Trial 1 using a DuPont Zorbax SAX column			
Apple	0.005	171, 181	137 ± 46 [34]
	1	95, 100	
Trial 1 using a Thomson Spherisorb SAX column			
Apple	0.005	199, 222	147 ± 74 [50]
	1	83, 85	
Trial 2 using a Thomson Spherisorb SAX column			
Apple	0.01	83, 85	87 ± 3 [3]
	1	89, 89	
Trial 2 using a Thomson Advantage SAX column			
Apple	0.01	82, 94	91 ± 6 [7]
	1	93, 94	

D. CONCLUSION

A successful ILV has been completed with apples using the HPLC method with fluorescence detection (Method NAA-AM-001). The HPLC method will be submitted to ACL/BEAD for regulatory agency validation (PMV) and to FDA for updating PAM II.

SECOND STUDY REPORT (OLIVES) :

44555403 Sterling, J. (1997) Independent Laboratory Validation of Analytical Method NAA-AM-002: Recommended Analytical Residue Method for 1-Naphthaleneacetic Acid, 1-Naphthaleneacetic, and/or 1-Naphthaleneacetic Acid Ethyl Ester, in Olives and Olive Matrices: Lab Project Number: AA970303: 4-97-3: NAA-AM-002. Unpublished study prepared by Southern Testing and Research labs., Inc. 71 p.

EXECUTIVE SUMMARY

Amvac Chemical Corporation has submitted the results of an independent laboratory validation (ILV) for a new analytical method, HPLC method NAA-AM-002 which quantitates residues of 1-naphthaleneacetic acid (NAA), 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester determined as the free acid (NAA) in olives and olive oil. The ILV study was conducted by Southern Testing and Research Laboratories, Inc. (Wilson, NC).

In brief, using Method NAA-AM-002 (similar to Method NAA-AM-001, discussed in detail above) residues of 1-naphthaleneacetic acid, 1-naphthalacetamide, and 1-naphthaleneacetic acid ethyl ester are extracted from olive and olive oil matricies by blending with deionized water. Residues are converted to the parent 1-naphthaleneacetic acid by base hydrolysis followed by partitioning with dichloromethane and a SPE-silica gel cleanup is performed prior to the determination of analyte concentration by HPLC using fluorescence detection. The column used was a Thomson Instrument Co. Advantage SAX 25 cm X 4.6 mm, with a Shimadzu HPLC system. Residues are calculated using linear regression from external standards of NAA and quantitated as the free acid (NAA), and a molecular weight conversion factor is applied to express residues in terms of the desired analyte (1-naphthalacetamide or 1-naphthaleneacetic acid ethyl ester). Samples were fortified with 1-naphthaleneacetic acid (NAA) at 0.001 and 1.0 ppm. For the initial trial with olives, recoveries ranged from 57 to 74% and averaged 64%. The trial was repeated using the same sample and spike levels and recoveries ranged from 71 to 86% and averaged 77%. Recoveries for olive oil ranged from 71 to 87% and averaged 79%. The method limit of quantitation (LOQ) is 0.01 ppm in/on olives and olive oil for each analyte in combination (expressed as NAA equivalents) or individually.

A successful ILV of Method NAA-AM-002 was completed. Acceptable recoveries were obtained from apples fortified with NAA at the method LOQ (0.01 ppm) and at 1.0 ppm. The subject method will be submitted to ACL/BEAD for additional regulatory method validation by Agency chemists and to FDA for updating PAM II.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D294853
PC Codes: 056002

Template Version September 2003

Naphthalene Acetates/PC Codes 056003, 056004, 056007, 056008/Amvac Chemical Corporation
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed - Olive

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03

STUDY REPORTS:

44555401 Singer, G. (1998) Magnitude of the Residue of Napthaleneacetic Acid in/on Olive Processed Commodities: Lab Project Number: AA960305: 4-97-4: AA960304. Unpublished study prepared by American Agricultural Services, Inc., Southern Testing and Research Laboratories, Inc. 228 p.

EXECUTIVE SUMMARY:

Amvac Chemical Company has submitted an olive processing study with NAA. Mature olives were harvested 176 days following the last of two sequential treatments consisting of a single spot treatment of the 1.15% ready-to-use (RTU) formulation (NAA ethyl ester) applied to runoff followed by a single broadcast thinning application of the 24.2% soluble concentrate (SC) formulation (NAA-potassium salt). The total application rates were 1.276 lb ai/A, 3.697 lb ai/A, or 6.148 lb ai/A (~1x, 3x, and 5x the application rates used in the crop field trials, respectively; see 860.1500 DER for MRID 44555402). Only samples of olives that were treated at 5x were processed into olive oil using simulated industrial processing procedures.

Samples of olives and its processed commodity (olive oil) were analyzed for residues of NAA using an HPLC method with fluorescence detection (Method NAA-AM-002). The validated LOQ was 0.01 ppm for olives. The maximum storage intervals of processing study samples were 296 days for olives and 283 days for olive oil. Adequate supporting storage stability data are available for olives but not for olive oil.

Three samples of 5x-treated olives bore NAA residues of 2.399, 2.668, and 2.899 ppm (average of 2.655 ppm) before processing. After processing, residues of NAA were 0.225, 0.255, and 0.318 ppm (average of 0.266 ppm) in olive oil indicating that residues of NAA reduced (0.1x) in olive oil. This observed processing factor does not exceed the theoretical concentration factor for olive oil. According to Table 3 of OPPTS 860.1520, the theoretical concentration factor is 108x for olive oil.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processing study residue data are classified as scientifically acceptable, pending submission of storage stability data for olive oil. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. NAA Nomenclature.

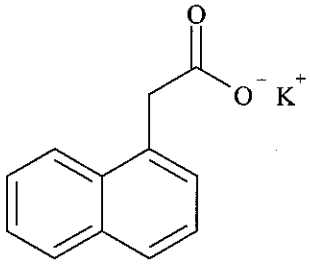
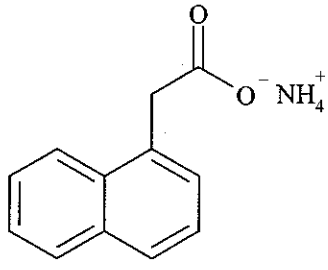
Chemical structure		
Common name	NAA potassium salt	NAA ammonium salt
Molecular Formula	$C_{12}H_{10}O_2K$	$C_{12}H_{13}NO_2$
Molecular Weight	225.31	203.24
IUPAC name	not available	not available
CAS name	1-naphthalnene acetic acid, potassium salt	1-naphthaleneacetic acid, ammonium salt
CAS #	15165-79-4	25545-89-5
PC Code	056003	056004
Current Food/Feed Site Registration	Apple, citrus hybrids other than tangelo, olive, orange, pear, tangelo, tangerine	Apple, cherry, olive, orange, pear, tangelo, and tangerine

TABLE A.1. NAA Nomenclature.

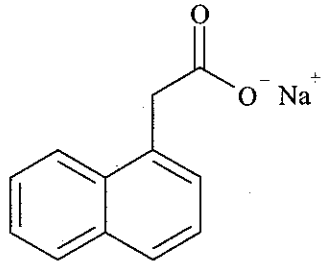
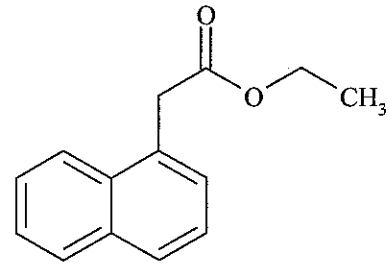
Chemical structure		
Common name	NAA sodium salt	NAA ethyl ester (NAA-OEt)
Molecular Formula	$C_{12}H_{10}O_2Na$	$C_{14}H_{14}O_2$
Molecular Weight	209.2	214.26
IUPAC name	not available	not available
CAS name	1-Naphthaleneacetic acid, sodium salt	1-Naphthaleneacetic acid, ethyl ester
CAS #	61-31-4	2122-70-5
PC Code	056007	056008
Current Food/Feed Site Registration	Apple, olive, pear	Apple, olive, pear

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
Active Ingredient	**NAA acetamide**	
Melting point/range	182-184 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	5.1	Product CSF
Density or specific gravity	0.221 g/cm^3	Product CSF
Water solubility (20°C)	not available	
Solvent solubility (20°C)	not available	
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA**	
Melting point/range	130 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	3.45	RD D265117, 5/15/00, B. Kitchens
Density or specific gravity	0.45 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	0.042 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	xylene 5.5 g/100 mL CCl_4 1.06 g/100 mL freely soluble in acetone, ether, and chloroform	CB Nos. 3468 and 3469, 6/3/88, F. Suhre Farm Chemicals Handbook

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]		
Parameter	**Value**	**Reference**
Vapor pressure at 20°C	0.3 mm Hg at 26 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA sodium salt**	
Melting point/range	>300 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
pH of 1% aqueous suspension	9.1	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Density or specific gravity	0.46 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	340 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	insoluble in nonpolar solvents	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA ethyl ester**	
Boiling point/range	>150 C	Old unreviewed Union Carbide data
pH of 1% aqueous suspension	not available	
Density or specific gravity	1.11 at 20 C	Old unreviewed Union Carbide data
Water solubility (26°C)	insoluble	Old unreviewed Union Carbide data
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	Old unreviewed Union Carbide data
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	

[1] No physicochemical properties information was available concerning the NAA potassium and ammonium salts.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.2. Study Use Pattern.

Location (County, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing, height	Vol (GPA[2])	Rate (lb ai/A)	RTI[3] (days)	Total Rate (lb ai/A)	
Tulare, CA; 1996 (1x plot)	1.15% RTU	1. Spot treatment; bloom. post pruning, 20 feet	Not applicable (NA)	0.246	22	1.276	NA
	24.2% SC [4]	2. Broadcast spray; immature fruit, 20 feet	859.6	1.03			spray mix prepared with a wetting agent
Tulare, CA; 1996 (3x plot)	1.15% RTU	1. Spot treatment; bloom. post pruning, 20 feet	NA	0.567	22	3.697	NA
	24.2% SC [4]	2. Broadcast spray; immature fruit, 20 feet	846.9	3.13			spray mix prepared with a wetting agent
Tulare, CA; 1996 (5x plot)	1.15% RTU	1. Spot treatment; bloom. post pruning, 20 feet	NA	0.848	22	6.148	NA
	24.2% SC [4]	2. Broadcast spray; immature fruit, 20 feet	857.3	5.3			spray mix prepared with a wetting agent

[1] EP = End-use Product
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
[4] We note that the registrant indicated that the test formulation (K-salt Fruit Fix 800; EPA Reg. No. 5481-130) is a NAA, potassium salt; however, according to the Agency's OPPIN database and PPLS, EPA Reg. No. 5481-130 is listed as a 21.4% SC formulation of NAA, ammonium salt (Fruit Fix Super Concentrate).

B.2. Sample Handling and Processing Procedures

Olive samples were harvested by hand at crop maturity and then frozen (-3 °C) within 3 hours of collection. A small RAC sample was shipped via Federal Express directly to Southern Testing and Research Laboratories, Inc. (Wilson, NC) for analysis. A larger bulk sample was shipped frozen via Federal Express to Food Protein Research & Development Center (Bryan, TX) for processing. Adequate descriptions of the processing procedures and material balance flowcharts were provided.

The RAC samples were processed into olive oil according to simulated industrial procedures within 38-58 days of harvest. Briefly, olives were washed and ground in a mill prior to crushing in a hydraulic press to separate the vegetable fluid and oil from the husk and seed. The vegetable fluid and oil were separated using a centrifuge and/or separatory funnel. The acid value of the oil

was determined, and the oil was identified as extra virgin oil, fine virgin oil, or virgin oil based on the acid value. All four processed samples were graded as extra virgin oil. Processed olive fractions were packed in dry ice and shipped via Federal Express to Southern Testing and Research Laboratories, Inc. (Wilson, NC) for analysis.

B.3. Analytical Methodology

Samples of olives and its processed commodity (olive oil) were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using an HPLC method with fluorescence detection (Method NAA-AM-002). Briefly, homogenized samples of olives and olive oil were blended with water, filtered, and boiled with 50% sodium hydroxide. The extract was filtered, washed with dichloromethane (DCM), and acidified to <pH 2 with concentrated hydrochloric acid. The acidified extract was then partitioned (2x) with DCM, and the resulting organic phase was dried over sodium sulfate and concentrated. An aliquot of the concentrate was purified through a silica gel solid-phase extraction (SPE-Si) cartridge; residues were eluted with DCM:acetic acid (99:1, v:v). The eluate was concentrated and redissolved in various mobile phases for HPLC analysis using a SAX column. The various mobile phases included the following: (i) for olives, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 10:90, v:v; (ii) for olive oil, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 20:80, v:v; and (iii) for 3 ppm olive spike study, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 20:80, v:v. The registrant found it necessary to adjust the composition of the mobile phase as the HPLC column aged in order to maintain comparable resolution and retention times. The validated limit of quantitation (LOQ) was 0.01 ppm for olives. Concurrent method recovery data (presented below in Table C.1) were submitted.

C. RESULTS AND DISCUSSION

Samples of olives and its processed commodity olive oil were frozen following processing until analysis. The maximum storage intervals of processing study samples from collection to analysis were 296 days for olives and 283 days for olive oil. Table C.2 lists the storage conditions and intervals of samples used in the olive processing study. Residues of NAA are relatively stable under frozen conditions for up to 365 days in olives, refer to the storage stability DER for MRID 44835301. No storage stability data are available for olive oil.

Samples of olives and its processed commodity (olive oil) were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using HPLC with fluorescence detection (Method NAA-AM-002). The validated LOQ was 0.01 ppm for olives. Concurrent recovery data included in the current submission indicate that the method is adequate for data collection in olives and olive oil. Apparent residues of NAA were below the LOQ in/on one sample each of untreated olive and olive oil.

Residue data from the 5x treatment plot (total rate of 6.148 lb ai/A) used in the olive processing study with NAA are reported in Table C.3. The registrant indicated that samples of olives and the resulting olive oil from plots treated at the 1x and 3x rates were not analyzed because the processing factor was readily determined from the olives treated at 5x. In the CA processing study reflecting the exaggerated 5x rate of the olive field trials (total rate of 6.148 lb ai/A), residues of NAA were 2.399, 2.668, and 2.899 ppm (average = 2.655 ppm) in/on the olive (RAC). Residues of NAA were 0.225, 0.255, and 0.318 ppm in olive oil indicating that residues of NAA reduced (0.1x) in olive oil. The reported processing factor does not exceed the theoretical concentration factor. According to Table 3 of OPPTS 860.1520, the theoretical concentration factor is 108x for olive oil.

TABLE C.1. Summary of Concurrent Recoveries of NAA from Olive and Olive Oil.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
NAA				
Olive	0.0122	1	113.11, 134.43 [1]	112.90 ± 33.2
	1.224	1	80.53, 80.55 [1]	
	3.075	1	115.97	
Olive oil	0.0098	1	90.82	93.42 ± 3.7
	0.9671	1	96.01	

[1] duplicate injections of a single sample.

TABLE C.2. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days) [1]
Olive	≤-10	296	Residues of NAA are relatively stable under frozen conditions for up to 365 days in olives. No storage stability data are available for olive oil.
Olive oil	≤-10	283	

[1] Refer to the DER for MRID 44835301.

TABLE C.3. Residue Data from Olive Processing Study with NAA.

RAC	Processed Commodity	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)	Processing Factor
Olive	RAC	0.848 + 5.3 = 6.148	176	2.399, 2.668, 2.899 (2.655)	--
	Olive oil			0.225, 0.255, 0.318	0.1x, 0.1x, 0.1x

D. CONCLUSION

The olive processing data indicate that residues of NAA do not concentrate in olive oil. Rather residues of NAA in olives were reduced (0.1x) in olive oil.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D294864
PC Codes: 056001, 056002, 056003, 056004, 056007, and 056008

Template Version September 2003

Naphthalene Acetates/PC Codes 056001, 056002, 056003, 056004, 056007, 056008/Amvac Chemical Corporation
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed - Apple

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03

STUDY REPORT:

44586501 Singer, G. (1997) Magnitude of the Residue of Naphthaleneacetic Acid in/on Processed Apple Commodities: Final Report: Lab Project Number: AA960304: 4-96-3: AA950304. Unpublished study prepared by American Agricultural Services, Inc. and Southern Testing and Research Labs., Inc. 212 p.

EXECUTIVE SUMMARY:

Amvac Chemical Company has submitted an apple processing study with NAA. Apples were harvested 2 days following the last of sequential treatments consisting of: (i) a single spot treatment of the 1.15% RTU formulation (NAA ethyl ester) to pruned areas or ground suckers; (ii) a single thinning application of the 8.4% WP formulation (NAA acetamide); and (iii) two preharvest drop applications of the 24.2% SC formulation (NAA-potassium salt). The registrant stated that the total application rates were made at 1x, 3x, or 5x the maximum label rates. Only samples of apples that were treated at 5x were processed into juice and wet pomace.

Samples of apples and its processed commodities were analyzed for residues of NAA using an HPLC method with fluorescence detection (Method NAA-AM-001). The validated LOQ was 0.01 ppm for apples. Method NAA-AM-001 is adequate for data collection based on acceptable concurrent method recoveries. The maximum storage intervals of processing study samples were 125 days for apples, 132 days for juice, and 143 days for wet pomace. Adequate supporting storage stability data are available for apples but not for apple processed commodities.

Residues of NAA in/on three apple samples that were treated at 5x were 0.092, 0.103, and 0.110 ppm (average of 0.102 ppm) before processing. After processing, residues of NAA concentrated 3.4x in wet apple pomace; no residue concentration was observed in apple juice (0.5x processing factor).

The maximum theoretical concentration factors for apples and apple pomace, listed in Tables 1 and 4 of OPPTS 860.1520, are >14x and 14x, respectively, and are experimental factors (based on comparison of proposed/established feed additive tolerances with proposed/established RAC tolerances); the apple pomace factor is most likely based on a tolerance for dry apple pomace, which is no longer regulated. The reported concentration factors do not exceed the theoretical concentration factors.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the apple processing data are classified as scientifically acceptable, pending submission of additional storage stability data for the processed commodities of apples (juice and wet pomace). The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. NAA Nomenclature.

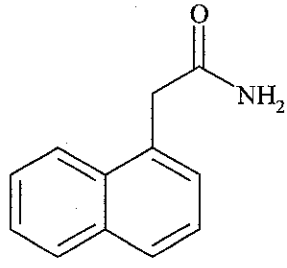
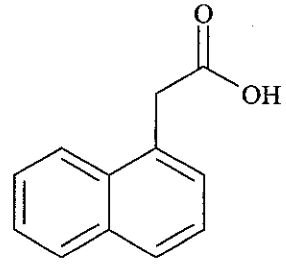
Chemical structure		
Common name	NAA acetamide (NAAm)	NAA
Molecular Formula	$C_{12}H_{11}NO$	$C_{12}H_{10}O_2$
Molecular Weight	185.23	186.20
IUPAC name	2-(1-naphthyl)acetamide	2-(1-naphthyl)acetic acid

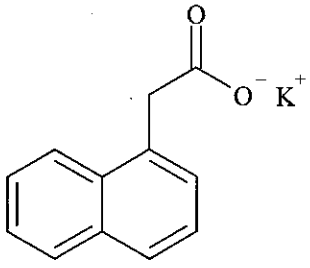
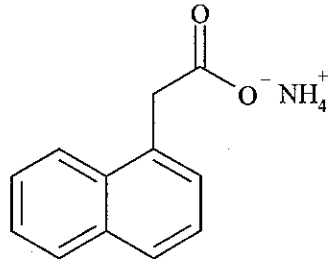
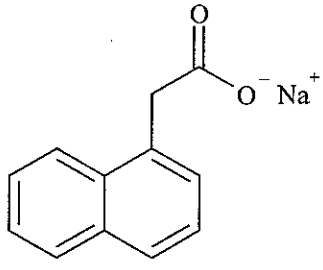
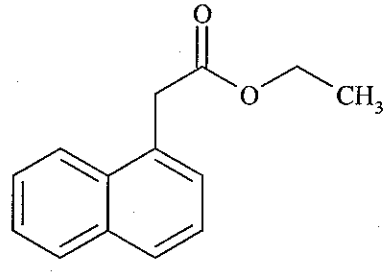
TABLE A.1. NAA Nomenclature.		
CAS name	1-naphthaleneacetamide	1-naphthaleneacetic acid
CAS #	86-86-2	86-87-3
PC Code	056001	056002
Current Food/Feed Site Registration	Apple, pear	Apple, pear
Chemical structure		
Common name	NAA potassium salt	NAA ammonium salt
Molecular Formula	$C_{12}H_{10}O_2K$	$C_{12}H_{13}NO_2$
Molecular Weight	225.31	203.24
IUPAC name	not available	not available
CAS name	1-naphthalnene acetic acid, potassium salt	1-naphthaleneacetic acid, ammonium salt
CAS #	15165-79-4	25545-89-5
PC Code	056003	056004
Current Food/Feed Site Registration	Apple, citrus hybrids other than tangelo, olive, orange, pear, tangelo, tangerine	Apple, cherry, olive, orange, pear, tangelo, and tangerine
Chemical structure		
Common name	NAA sodium salt	NAA ethyl ester (NAA-OEt)
Molecular Formula	$C_{12}H_{10}O_2Na$	$C_{14}H_{14}O_2$
Molecular Weight	209.2	214.26
IUPAC name	not available	not available
CAS name	1-Naphthaleneacetic acid, sodium salt	1-Naphthaleneacetic acid, ethyl ester
CAS #	61-31-4	2122-70-5
PC Code	056007	056008
Current Food/Feed Site Registration	Apple, olive, pear	Apple, olive, pear

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
Active Ingredient	**NAA acetamide**	
Melting point/range	182-184 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	5.1	Product CSF
Density or specific gravity	0.221 g/cm^3	Product CSF
Water solubility (20°C)	not available	
Solvent solubility (20°C)	not available	
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA**	
Melting point/range	130 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	3.45	RD D265117, 5/15/00, B. Kitchens
Density or specific gravity	0.45 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	0.042 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	xylene 5.5 g/100 mL CCl_4 1.06 g/100 mL freely soluble in acetone, ether, and chloroform	CB Nos. 3468 and 3469, 6/3/88, F. Suhre Farm Chemicals Handbook
Vapor pressure at 20°C	0.3 mm Hg at 26 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA sodium salt**	
Melting point/range	>300 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
pH of 1% aqueous suspension	9.1	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Density or specific gravity	0.46 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	340 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	insoluble in nonpolar solvents	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
Active ingredient	**NAA ethyl ester**	
Boiling point/range	>150 C	Old unreviewed Union Carbide data
pH of 1% aqueous suspension	not available	
Density or specific gravity	1.11 at 20 C	Old unreviewed Union Carbide data
Water solubility (26°C)	insoluble	Old unreviewed Union Carbide data
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	Old unreviewed Union Carbide data
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	

[1] No physicochemical properties information was available concerning the NAA potassium and ammonium salts.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.2. Study Use Pattern.

Location (County, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing, height	Vol (GPA[2])	Rate	RTI[3] (days)	Total Rate (lb ai/A) [4]	
Wayne, NY; 1996 (1x plot)	1.15% RTU	1. Spot treatment; after pruning, 10 feet	Not applicable (NA)	1.33 g ai	--	Not calculated	NA
	8.4% WP	2. Broadcast spray; 4 weeks past full bloom, 10 feet	150.9	51.6 ppm	11		Not specified (NS)
	24.2% SC [5]	3. Broadcast spray; colored fruit, 10 feet	150.2	5.6 fl.oz product/A	96		NS
		4. Broadcast spray; 6.3-7.6 cm diameter fruit, 10 feet	150.8	5.6 fl.oz product/A	8		NS
Wayne, NY; 1996 (3x plot)	1.15% RTU	1. Spot treatment; after pruning, 10 feet	NA	2.76 g ai	--	Not calculated	NA
	8.4% WP	2. Broadcast spray; 4 weeks past full bloom, 10 feet	151.1	154 ppm	11		NS
	24.2% SC [5]	3. Broadcast spray; colored fruit, 10 feet	150.2	16.8 fl.oz product/A	96		NS
		4. Broadcast spray; 6.3-7.6 cm diameter fruit, 10 feet	150.7	16.8 fl.oz product/A	8		NS
Wayne, NY; 1996 (5x plot)	1.15% RTU	1. Spot treatment; after pruning, 10 feet	NA	3.97 g ai	--	Not calculated	NA
	8.4% WP	2. Broadcast spray; 4 weeks past full bloom, 10 feet	150.4	257.6 ppm	11		NS
	24.2% SC [5]	3. Broadcast spray; colored fruit, 10 feet	150.5	28.0 fl.oz product/A	96		NS
		4. Broadcast spray; 6.3-7.6 cm diameter fruit, 10 feet	150.4	28.0 fl.oz product/A	8		NS

[1] EP = End-use Product
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval
[4] The study reviewer could not calculate the total rate.
[5] We note that the registrant indicated that the test formulation (K-salt Fruit Fix 800; EPA Reg. No. 5481-130) is a NAA, potassium salt; however, according to the Agency's OPPIN database and PPLS, EPA Reg. No. 5481-130 is listed as a 21.4% SC formulation of NAA, ammonium salt (Fruit Fix Super Concentrate).

B.2. Sample Handling and Processing Procedures

A single bulk sample (100 lb) of apple fruit was collected from the control and treated plots. In addition, a small RAC sample (5 lb) was stored frozen at the field site for 8 days prior to shipment to Southern Testing and Research Laboratories, Inc. (Wilson, NC) for analysis. The bulk sample of apple fruit was hand-delivered at ambient temperatures to A.C.D.S. Research, Inc. (Lyons, NY) on the day of field collection for processing.

Samples were processed according to simulated commercial procedures within 1 day of harvest into juice and wet pomace. Apples were ground in a hammermill, and the resulting wet mash was loaded, in portions, into cloth stacks, pressed, and the juice was collected in plastic bottles. After the pressing cycle, the wet pomace cake was broken up to obtain a sample (wet pomace). Adequate descriptions of the processing procedures and material balance flowcharts were provided. Processed apple fractions were immediately frozen and shipped to Southern Testing and Research Laboratories, Inc. (Wilson, NC) for analysis.

B.3. Analytical Methodology

Samples of apples and its processed commodities (juice and wet pomace) were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using an HPLC method with fluorescence detection (Method NAA-AM-001). Briefly, homogenized samples of apple matrices were blended with water and a 50% sodium hydroxide solution was added so that the resulting mixture contains at least 10% sodium hydroxide. An antifoam agent was added to prevent excessive bumping during hydrolysis at reflux for 3 hours. The extract was vacuum filtered and partitioned with dichloromethane (DCM). The organic phase was discarded and the aqueous phase was acidified to <pH 2 with concentrated hydrochloric acid. The acidic extract was then partitioned (2x) with dichloromethane. The resulting organic phase was collected through sodium sulfate and concentrated by rotary evaporation; the volume was adjusted with dichloromethane. An aliquot of the concentrate was purified through a silica gel solid-phase extraction (SPE-Si) cartridge; residues were eluted with DCM:acetic acid (99:1, v:v) and subjected to solvent exchange to be suitable for HPLC analysis. An aliquot of the SPE-Si eluate is concentrated, acetonitrile (ACN) is added, and the eluate concentrated again. The concentrated residues are then diluted with ACN, and water is added. Residues in ACN:water (1:1, v:v) are filtered prior to analysis by HPLC with fluorescence detection. The validated limit of quantitation (LOQ) was 0.01 ppm for apples. The registrant reported NAA residues as <0.05 ppm for controls in summary table on page 13 of submission, but fortified at 0.01 and 0.1 ppm for concurrent recovery.) Concurrent method recovery data (presented below in Table C.1) were submitted.

C. RESULTS AND DISCUSSION

Samples of apples and its processed commodities juice and wet pomace were frozen following processing until analysis. The maximum storage intervals of processing study samples from collection to analysis were 125 days for apples, 132 days for juice, and 143 days for wet pomace. Table C.2 lists the storage conditions and intervals of samples used in the apple processing study. Residues of NAA are relatively stable under frozen conditions for up to 12 months in apples, refer to the storage stability DER for MRIDs 44660201 and 44660202. No storage stability data are available for processed commodities (apple juice and wet pomace).

Samples of apples and its processed commodities (apple juice and wet pomace) were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using HPLC with fluorescence detection (Method NAA-AM-001). The validated LOQ was 0.01 ppm for apples. Concurrent recovery data included in the current submission indicate that the method is adequate for data collection in apples and its processed commodities juice and wet pomace. Apparent residues of NAA were <0.05 ppm in/on three samples each of untreated apples, juice, and wet pomace.

Residue data from the 5x treatment plot used in the apple processing study with NAA are reported in Table C.3. The registrant indicated that samples of apples and the resulting juice and wet pomace from plots treated at the 1x and 3x rates were not analyzed because the residue levels from these treatments were expected to be too low to provide reliable residues and processing factors. In the CA processing study reflecting the exaggerated 5x rate, residues of NAA were 0.092, 0.103, and 0.110 ppm (average = 0.102 ppm) in/on the apple fruit (RAC) from the processor. The processing data for apple juice and wet pomace indicate that residues of NAA concentrate in wet apple pomace (3.4x processing factor) but do not concentrate in apple juice (0.5x processing factor) processed from apples bearing detectable residues of NAA.

TABLE C.1. Summary of Concurrent Recoveries of NAA from Apple and its Processed Commodities (Juice and Pomace).

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Apple	0.0116, 0.0118	2	72, 73	82.4 ± 14.5
	0.1178, 0.1182	2	94, 71	
	0.2341	1	102	
Juice	0.0116	1	84	89.5
	0.118	1	95	
Pomace	0.0117	1	102	97
	0.4702	1	92	

TABLE C.2. Summary of Storage Conditions			
Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration from Collection (processing) to Analysis (days) [1]	Interval of Demonstrated Storage Stability (days) [2]
Apple (RAC; field)	≤-10	117	Residues of NAA are relatively stable under frozen conditions for up to 12 months in apples. No storage stability data are available for processed commodities apple juice and wet pomace.
Apple (RAC; processor)	≤-10	125	
Juice	≤-10	132	
Pomace	≤-10	137 143, reanalysis	

[1] Apple samples were processed within 1 day of harvest.
[2] Refer to the DER for MRIDs 44660201 and 44660202.

TABLE C.3. Residue Data from Apple Processing Study with NAA.					
RAC	Processed Commodity	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)	Processing Factor
Apple	RAC (field)	Not calculated (5x treatment plot)	2	0.1635, 0.1693, 0.1776 (0.1701)	--
	RAC (processor)			0.0924, 0.1032, 0.1103 (0.1020)	--
	Juice			0.0502, 0.0516, 0.0535	0.5x, 0.5x, 0.5x (0.5x)
	Pomace			0.3178, 0.3490, 0.3663	3.1x, 3.4x, 3.6x (3.4x)

D. CONCLUSION

The processing data indicate that residues of NAA may concentrate by an average concentration factor of 3.4x in wet apple pomace but do not concentrate in apple juice (0.5x processing factor) processed from apples bearing detectable residues of NAA.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D294864
PC Codes: 056001, 056002, 056003, 056004, 056007, and 056008

Template Version September 2003

Naphthalene Acetates/PC Codes 056001, 056002, 056003, 056004, 056007, 056008/Amvac Chemical Corporation
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Apple and Pear

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03

STUDY REPORTS:

44660201 Hathaway, M. (1998) Frozen Storage Stability of Naphthaleneacetic acid in Apples: Lab Project Number: AA970301: 4-97-1: NAA-AM-001. Unpublished study prepared by Southern Testing and Research Laboratories, Inc. 78 p.

44660202 Hathaway, M. (1998) Frozen Storage Stability of Naphthaleneacetic acid in Pears: Lab Project Number: AA970302: 4-97-2: NAA-AM-001. Unpublished study prepared by Southern Testing and Research Laboratories, Inc. 79 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted the results of two studies which investigated the storage stability of 1-naphthaleneacetic acid (NAA) in/on apples and pears. Untreated samples of these fruits were fortified with NAA at a nominal level of 0.25 ppm. The fortified samples were stored frozen (-20 to -10 °C) for a duration of 363-364 days (12 months) then analyzed for residues of NAA using an HPLC method with fluorescence detection (Method NAA-AM-001). The data-collection method is adequate based on acceptable concurrent method recoveries. The results of the current studies indicate that residues of NAA are reasonably stable under frozen storage conditions in/on apples and pears for up to 12 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. NAA Nomenclature.

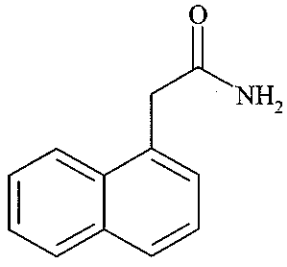
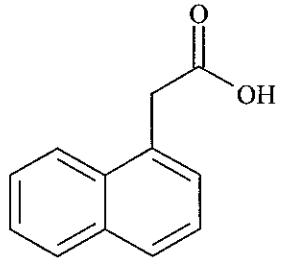
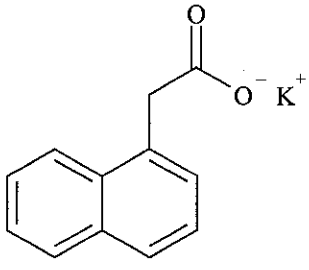
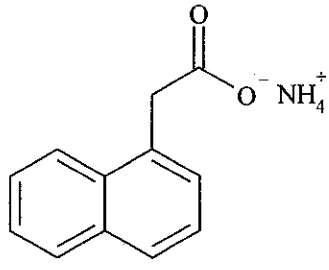
Chemical structure		
Common name	NAA acetamide (NAAm)	NAA
Molecular Formula	$C_{12}H_{11}NO$	$C_{12}H_{10}O_2$
Molecular Weight	185.23	186.20
IUPAC name	2-(1-naphthyl)acetamide	2-(1-naphthyl)acetic acid
CAS name	1-naphthaleneacetamide	1-naphthaleneacetic acid
CAS #	86-86-2	86-87-3
PC Code	056001	056002
Current Food/Feed Site Registration	Apple, pear	Apple, pear
Chemical structure		

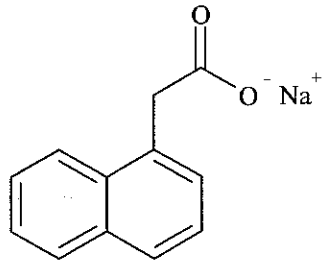
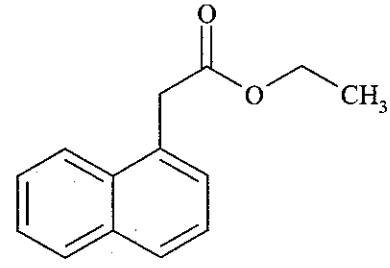
TABLE A.1. NAA Nomenclature.		
Common name	NAA potassium salt	NAA ammonium salt
Molecular Formula	$C_{12}H_{10}O_2K$	$C_{12}H_{13}NO_2$
Molecular Weight	225.31	203.24
IUPAC name	not available	not available
CAS name	1-naphthalnene acetic acid, potassium salt	1-naphthaleneacetic acid, ammonium salt
CAS #	15165-79-4	25545-89-5
PC Code	056003	056004
Current Food/Feed Site Registration	Apple, citrus hybrids other than tangelo, olive, orange, pear, tangelo, tangerine	Apple, cherry, olive, orange, pear, tangelo, and tangerine
Chemical structure		
Common name	NAA sodium salt	NAA ethyl ester (NAA-OEt)
Molecular Formula	$C_{12}H_{10}O_2Na$	$C_{14}H_{14}O_2$
Molecular Weight	209.2	214.26
IUPAC name	not available	not available
CAS name	1-Naphthaleneacetic acid, sodium salt	1-Naphthaleneacetic acid, ethyl ester
CAS #	61-31-4	2122-70-5
PC Code	056007	056008
Current Food/Feed Site Registration	Apple, olive, pear	Apple, olive, pear

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]		
Parameter	**Value**	**Reference**
Active Ingredient	**NAA acetamide**	
Melting point/range	182-184 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	5.1	Product CSF
Density or specific gravity	0.221 g/cm^3	Product CSF
Water solubility (20°C)	not available	
Solvent solubility (20°C)	not available	
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
UV/vis absorption spectrum	not available	

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
Active ingredient	**NAA**	
Melting point/range	130 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	3.45	RD D265117, 5/15/00, B. Kitchens
Density or specific gravity	0.45 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	0.042 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	xylene 5.5 g/100 mL CCl_4 1.06 g/100 mL freely soluble in acetone, ether, and chloroform	CB Nos. 3468 and 3469, 6/3/88, F. Suhre Farm Chemicals Handbook
Vapor pressure at 20°C	0.3 mm Hg at 26 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA sodium salt**	
Melting point/range	>300 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
pH of 1% aqueous suspension	9.1	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Density or specific gravity	0.46 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	340 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	insoluble in nonpolar solvents	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA ethyl ester**	
Boiling point/range	>150 C	Old unreviewed Union Carbide data
pH of 1% aqueous suspension	not available	
Density or specific gravity	1.11 at 20 C	Old unreviewed Union Carbide data
Water solubility (26°C)	insoluble	Old unreviewed Union Carbide data
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	Old unreviewed Union Carbide data
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	

[1] No physicochemical properties information was available concerning the NAA potassium and ammonium salts.

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

Untreated samples of apples and pears were fortified with NAA at a nominal concentration of 0.25 ppm (actual concentrations ranged from 0.239-0.257 ppm in apples and 0.239-0.243 ppm in pears). The NAA fortification standard was prepared in acetonitrile (ACN). The fortified samples were then stored frozen at -20 to -10 °C. A complete description of the spiking procedure, including the stability of the standard spiking solution, the condition of the matrix at the time of spiking, and the time allowed for equilibrium was not provided.

The registrant indicated that for 4 days in June, the temperature in the freezer used to store the stability samples deviated from -20 to -10 °C due to a freon compressor malfunction. As a result, the temperature inside the freezer reached 9 °C. To ascertain if any negative effect was incurred as a result of the freezer malfunction, three reserved spiked apple samples (0.239-0.243 ppm) were analyzed four days later. Recoveries of the three samples ranged 84.1-106%.

B.2. Analytical Methodology

Samples of apples and pears were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using an HPLC method with fluorescence detection (Method NAA-AM-001). The method LOQ was 0.01 ppm for NAA. Concurrent method recovery data (presented below in Table C.1) were submitted.

Briefly, homogenized samples of apple and pear were blended with water and a 50% sodium hydroxide solution was added so that the resulting mixture contains at least 10% sodium hydroxide. An antifoam agent was added to prevent excessive bumping during hydrolysis at reflux for 3 hours. The extract was vacuum filtered and partitioned with dichloromethane (DCM). The organic phase was discarded, and the aqueous phase was acidified to <pH 2 with concentrated hydrochloric acid. The acidic extract was then partitioned (2x) with dichloromethane. The resulting organic phase was collected through sodium sulfate and concentrated by rotary evaporation; the volume was adjusted with dichloromethane. An aliquot of the concentrate was purified through a silica gel solid-phase extraction (SPE-Si) cartridge; residues were eluted with DCM:acetic acid (99:1, v:v) and subjected to solvent exchange to be suitable for HPLC analysis. An aliquot of the SPE-Si eluate was concentrated, acetonitrile (ACN) was added, and the eluate concentrated again. The concentrated residues were then diluted with ACN, and water was added. Residues in ACN:water (1:1, v:v) were filtered prior to analysis by HPLC with fluorescence detection.

C. RESULTS AND DISCUSSION

The concurrent method validation data indicate that Method NAA-AM-001 is adequate for the determination of residues of NAA in/on apple and pear fruit.

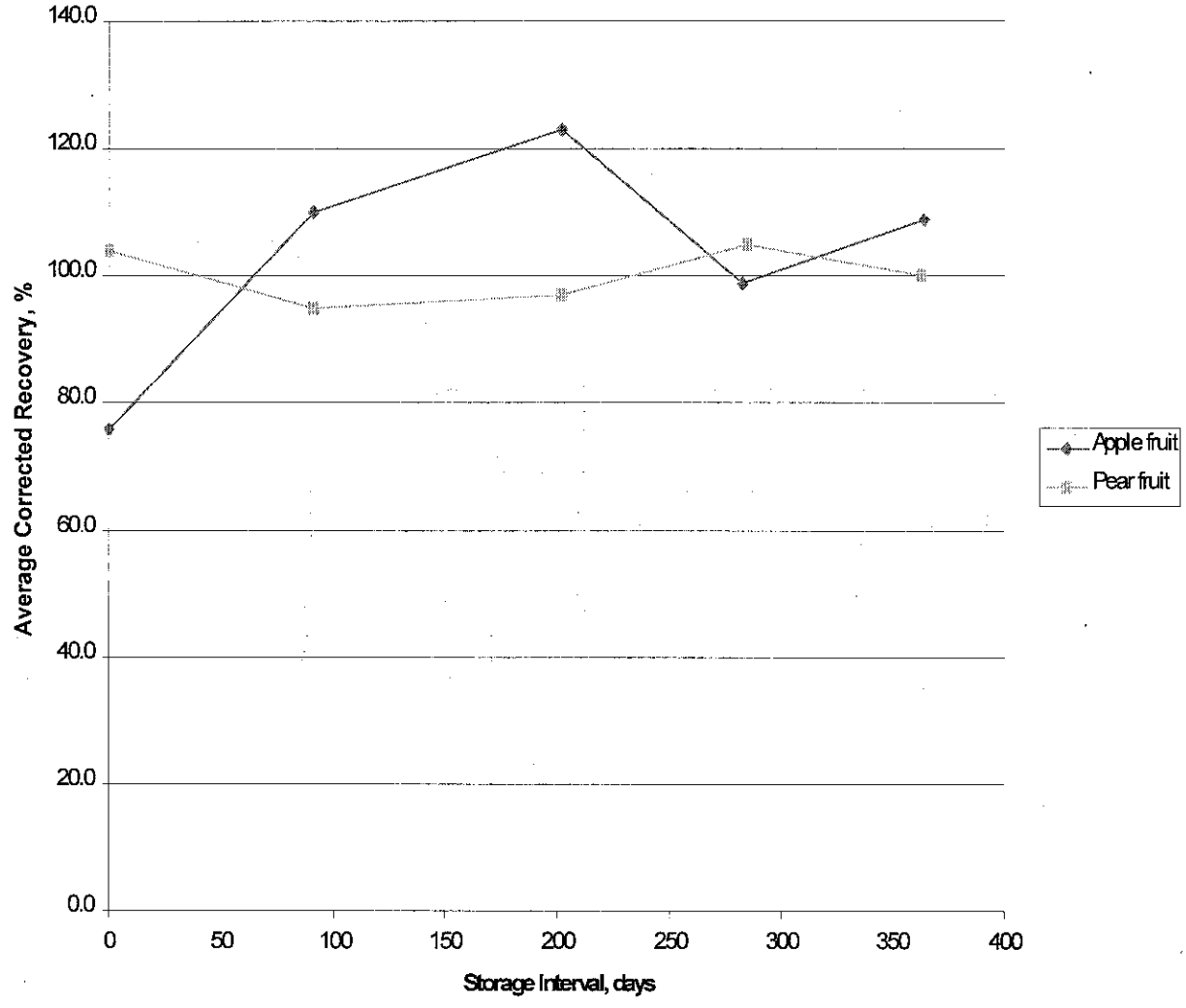
The submitted storage stability data indicate that residues of NAA are stable in/on apples and pears for up to 363-364 days (12 months) of frozen storage.

TABLE C.1. Summary of Concurrent Recoveries of NAA from Apple and Pear.

Matrix	Spike level (ppm) [1]	Storage Interval (days)	Sample size (n)	Recoveries (%)	Mean ± std dev
NAA					
Apple	0.239-0.257	0	3	73.8, 75.3, 78.2	75.8 ± 2.2
		91	2	87.4, 91.4	89.4
		202	2	85.2, 93.9	89.6
		283	2	96.4, 100	98.2
		364	2	75.5, 77.6	109
Pear	0.239-0.243	0	3	102, 104, 107	104 ± 2.5
		91	2	93.0, 102	97.5
		202	2	106, 107	107
		285	2	103, 106	105
		363	2	88.8, 92.0	90.4

[1] Samples were fortified a nominal concentration of 0.25 ppm (actual concentrations ranged from 0.239-0.257 ppm, depending on the actual spike sample weight used).

FIGURE C.1. Graph of Storage Stability of NAA in Apple and Pear Fruit.



C.2. Stability of NAA Residues in Apple and Pear Following Storage at -20 to -10 °C.				
Commodity	Spike level (ppm) [1]	Storage interval (days)	Recovered residues (ppm)	Corrected % recovery [2]
Apple	0.239-0.257	0	0.177, 0.183, 0.190	75.8
		91	0.224, 0.235, 0.252	110
		202	0.229, 0.274, 0.289	123
		283	0.215, 0.235, 0.252	98.9
		364	0.191, 0.200, 0.210	109
Pear	0.239-0.243	0	0.248, 0.253, 0.257	104
		91	0.201, 0.224, 0.245	94.8
		202	0.250, 0.252, 0.253	97.2
		285	0.243, 0.255, 0.258	105
		363	0.218, 0.218, 0.218	100

[1] Samples were fortified a nominal concentration of 0.25 ppm (actual concentrations ranged from 0.239-0.257 ppm, depending on the actual spike sample weight used).
[2] Average corrected recoveries as calculated by the registrant using actual fortification levels (0.239-0.257 ppm) were reported. Individual fortification levels were not provided.

D. CONCLUSION

The submitted storage stability data indicate that residues of NAA are stable in/on apple and pear fruits for up to 12 months of frozen storage.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D295126
PC Codes: 056001, 056002, 056003, 056004, 056007, and 056008

Template Version September 2003

Naphthalene Acetates/PC Codes 056003, 056004, 056007, 056008/Amvac Chemical Corporation
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Olive

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 11/06/03

STUDY REPORT:

44555402 Singer, G. (1998) Magnitude of the Residue of Napthaleneacetic Acid in/on Olive Raw Agricultural Commodities: Lab Project Number: AA960306: 4-97-5. Unpublished study prepared by American Agricultural Services, Inc., Southern Testing and Research Labs., Inc. 136 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted data depicting the magnitude of 1-naphthaleneacetic acid (NAA) in/on olives. Three olive field trials were conducted in Region 10 (CA) during the 1996 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, olives were harvested 102-112 days following the last of two sequential treatments consisting of: (i) a single spot treatment of the 1.15% ready-to-use (RTU) formulation (NAA ethyl ester) applied to runoff at a rate of 0.14-1.00 lb ai/A to control suckers and sprouting early in the growing season; and (ii) a single broadcast thinning application of the 24.2% soluble concentrate (SC) formulation (NAA-potassium salt) at 0.871-1.13 lb ai/A.

Samples of olives were analyzed for residues of NAA using an HPLC method with fluorescence detection (Method NAA-AM-002). The validated LOQ was 0.01 ppm for olives. Concurrent recovery data indicate that the method is adequate for data collection. The maximum storage interval of olive samples, that were collected from the current study, was 308 days. In support of the olive field trials, the registrant submitted storage stability data (refer to the DER for MRID 44835301) which demonstrated that residues of NAA are relatively stable under frozen storage conditions for up to 365 days in/on olives.

The maximum residue of NAA in/on treated olives from the submitted olive field trials was 0.610 ppm.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the olive residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. NAA Nomenclature.

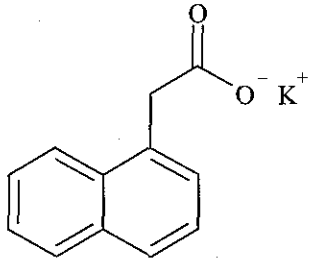
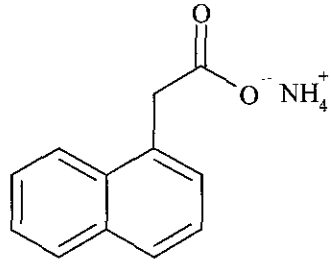
Chemical structure		
Common name	NAA potassium salt	NAA ammonium salt
Molecular Formula	$C_{12}H_{10}O_2K$	$C_{12}H_{13}NO_2$
Molecular Weight	225.31	203.24
IUPAC name	not available	not available
CAS name	1-naphthalnene acetic acid, potassium salt	1-naphthaleneacetic acid, ammonium salt
CAS #	15165-79-4	25545-89-5
PC Code	056003	056004
Current Food/Feed Site Registration	Apple, citrus hybrids other than tangelo, olive, orange, pear, tangelo, tangerine	Apple, cherry, olive, orange, pear, tangelo, and tangerine

TABLE A.1. NAA Nomenclature.

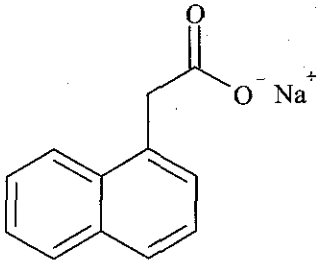
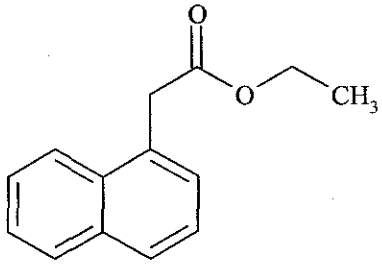
Chemical structure		
Common name	NAA sodium salt	NAA ethyl ester (NAA-OEt)
Molecular Formula	$C_{12}H_{10}O_2Na$	$C_{14}H_{14}O_2$
Molecular Weight	209.2	214.26
IUPAC name	not available	not available
CAS name	1-Naphthaleneacetic acid, sodium salt	1-Naphthaleneacetic acid, ethyl ester
CAS #	61-31-4	2122-70-5
PC Code	056007	056008
Current Food/Feed Site Registration	Apple, olive, pear	Apple, olive, pear

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]

Parameter	Value	Reference
Active Ingredient	**NAA acetamide**	
Melting point/range	182-184 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	5.1	Product CSF
Density or specific gravity	0.221 g/cm^3	Product CSF
Water solubility (20°C)	not available	
Solvent solubility (20°C)	not available	
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA**	
Melting point/range	130 C	Farm Chemicals Handbook
pH of 1% aqueous suspension	3.45	RD D265117, 5/15/00, B. Kitchens
Density or specific gravity	0.45 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	0.042 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	xylene 5.5 g/100 mL CCl_4 1.06 g/100 mL freely soluble in acetone, ether, and chloroform	CB Nos. 3468 and 3469, 6/3/88, F. Suhre Farm Chemicals Handbook

TABLE A.2. Physicochemical Properties of the Technical Grade NAA. [1]		
Parameter	**Value**	**Reference**
Vapor pressure at 20°C	0.3 mm Hg at 26 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA sodium salt**	
Melting point/range	>300 C	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
pH of 1% aqueous suspension	9.1	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Density or specific gravity	0.46 g/mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Water solubility (26°C)	340 g/100 mL	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Solvent solubility (26°C)	insoluble in nonpolar solvents	CB Nos. 3468 and 3469, 6/3/88, F. Suhre
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	3.16×10^{-4}	CB Nos. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient (K_{ow})	not applicable; polar compound	
UV/vis absorption spectrum	not available	
Active ingredient	**NAA ethyl ester**	
Boiling point/range	>150 C	Old unreviewed Union Carbide data
pH of 1% aqueous suspension	not available	
Density or specific gravity	1.11 at 20 C	Old unreviewed Union Carbide data
Water solubility (26°C)	insoluble	Old unreviewed Union Carbide data
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	Old unreviewed Union Carbide data
Vapor pressure at 20°C	not available	
Dissociation constant (pK_a)	not available	
Octanol/water partition coefficient (K_{ow})	not available	
UV/vis absorption spectrum	not available	

[1] No physicochemical properties information was available concerning the NAA potassium and ammonium salts.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions.

Trial Identification (County, State; Year)	Soil characteristics				Meteorological data	
	Type	%OM*	pH*	CEC* (meq/g)	Overall monthly rainfall range (inches)	Overall temperature range (°C)
Tulare, CA; 1996 (AA960306.CA1)	Soil type was not provided; Not Applicable.				0.00	13.3-38.9
Kern, CA; 1996 (AA960306.CA2)	Soil type was not provided; Not Applicable.				0.00	11.1-37.2
Tulare, CA: 1996 (AA960306.CA3)	Soil type was not provided; Not Applicable.				0.00	13.3-38.9

*These parameters are optional except in cases where their value affects the use pattern for the chemical.

The actual temperature recordings are within average historical values for the residue study period. The actual rainfall average was also within the historical rainfall average. Irrigation was used to supplement as needed. The registrant indicated that weather data demonstrate that climatological conditions during the study were typical for the region and did not alter the normal growth, development, or maturity of the olives at the test sites.

TABLE B.1.2. Study Use Pattern.

Location (City, State; Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing, height	Vol. (GPA[2])	Rate (lb ai/A)	RTI[3] (days)	Total Rate (lb ai/A)	
Tulare, CA; 1996 (AA960306.CA1)	1.15% RTU	1. Spot treatment; bloom, 20 feet	Not applicable (NA)	0.27	18	0.27 + 1.00 = 1.27	NA
	24.2% SC[4]	2. Broadcast spray; early fruit set, 20 feet	835.3	1.00			spray mix prepared without oil but with a wetting agent
Kern, CA; 1996 (AA960306.CA2)	1.15% RTU	1. Spot treatment; flowering, 20 feet	NA	0.14	18	0.14 + 1.13 = 1.27	NA
	24.2% SC	2. Broadcast spray; immature fruit, 20 feet	911.6	1.13			spray mix prepared without oil but with a wetting agent
Tulare, CA; 1996 (AA960306.CA3)	1.15% RTU	1. Spot treatment; bloom, 20 feet	NA	1.0	17	1.0 + 0.871 = 1.871	NA
	24.2% SC	2. Broadcast spray; early fruit set, 20 feet	725.6	0.871			spray mix prepared without oil but with a wetting agent

[1] EP = End-use Product; two sequential applications were made with a 1.15% ready to use (RTU) formulation of ethyl 1-naphthaleneacetate (EPA Reg. No. 5481-452) followed by a 24.2% soluble concentrate (SC) formulation of 1-naphthaleneacetic acid, potassium salt (EPA Reg. No. 5481-130).
[2] GPA = Gallons per acre
[3] RTI = Retreatment Interval

[4] We note that the registrant indicated that the 24.2% SC formulation (K-salt Fruit Fix 800; EPA Reg. No. 5481-130) is a potassium salt of NAA; however, according to the Agency's OPPIN database and PPLS, EPA Reg. No. 5481-130 is listed as a 21.4% SC formulation of NAA, ammonium salt (Fruit Fix Super Concentrate).

TABLE B.1.3. *Trial Numbers and Geographical Locations.*

	Olive		
		Requested [1]	
NAFTA Growing Region	Submitted	Canada	US
1			
1A			
2			
3			
4			
5			
5A			
5B			
6			
7			
7A			
8			
9			
10	3		
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
Total	3		

[1] A total of 3 trials are requested for olives; geographic distribution of field trials for olives is not specified for crops requiring ≤3 trials.

B.2. Sample Handling and Preparation

Olive samples were harvested by hand at crop maturity, frozen (-23 to -15 °C) within 1.17 hours of collection, and shipped via Federal Express to Southern Testing and Research Laboratories, Inc. (Wilson, NC) for analysis.

B.3. Analytical Methodology

Samples of olives were analyzed for residues of NAA by Southern Testing and Research Laboratories, Inc. (Wilson, NC) using an HPLC method with fluorescence detection (Method NAA-AM-002). Briefly, homogenized samples of olives were blended with water, filtered, and boiled with 50% sodium hydroxide. The extract was filtered, washed with dichloromethane (DCM), and acidified to <pH 2 with concentrated hydrochloric acid. The acidified extract was then partitioned (2x) with DCM, and the resulting organic phase was dried over sodium sulfate and concentrated. An aliquot of the concentrate was purified through a silica gel solid-phase extraction (SPE-Si) cartridge; residues were eluted with DCM:acetic acid (99:1, v:v). The eluate was concentrated and redissolved in various mobile phases for HPLC analysis using a SAX column. The various mobile phases included the following: (i) for Trial1, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 40:60, v:v; (ii) for the rerun of Trial 1, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 35:65, v:v; (iii) for Trials 2 and 3, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 35:65, v:v; and (iv) for the rerun of Trials 2 and 3, the mobile phase was ACN:0.05 M KH_2PO_4, pH 5.0; 25:75, v:v. The registrant found it necessary to adjust the composition of the mobile phase as the HPLC column aged in order to maintain comparable resolution and retention times. The validated limit of quantitation (LOQ) was 0.01 ppm for olives. Concurrent method recovery data (presented below in Table C.1) were submitted.

C. RESULTS AND DISCUSSION

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage intervals of crop samples from harvest to analysis were 308 days for olives. In support of the olive field trials, the registrant submitted storage stability data (refer to the DER for MRID 44835301) which demonstrated that residues of NAA are relatively stable for up to 365 days in/on olives.

Samples of olives were analyzed for residues of NAA using Method NAA-AM-002. The validated LOQ was 0.01 ppm for olives. Concurrent recovery data included in the current submission indicate that the method is adequate for data collection in olives.

Residue data from the olive field trials with NAA are reported in Table C.3. A summary of residue data for olives following sequential treatments with the 1.15% RTU and the 24.2% SC formulations is presented in Table C.4. Residues of NAA were 0.306-0.610 ppm in/on olives harvested 102-112 days following the last of two sequential treatments consisting of (i) a single spot treatment of the 1.15% RTU formulation (NAA ethyl ester; Product Name: Tre-Hold RTU Sprout Inhibitor; EPA Reg. No. 5481-452) applied to runoff at a rate of 0.14-1.00 lb ai/A to

control suckers and sprouting early in the growing season; and (ii) a single broadcast thinning application of the 24.2% SC formulation (NAA-potassium salt; Product Name: K-salt Fruit Fix 800; EPA Reg. No. 5481-130) at 0.871-1.13 lb ai/A. We note that the registrant indicated that the 24.2% SC formulation (K-salt Fruit Fix 800; EPA Reg. No. 5481-130) is a 1-napthaleneacetic acid, potassium salt; however, according to the Agency's OPPIN database and PPLS, EPA Reg. No. 5481-130 is listed as a 24.1% SC formulation of 1-napthaleneacetic acid, ammonium salt (Fruit Fix Super Concentrate).

Apparent residues of NAA were less than the method LOQ (<0.01 ppm) in/on 3 untreated samples of olives.

TABLE C.1. Summary of Concurrent Recoveries of NAA from Olives.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
NAA				
Olive	0.01	2	58.00, 119.0 [1]	89.6 ± 19.5
	1.00	4	85.42, 90.67, 91.58, 93.15	

[1] Four samples fortified at 0.01 ppm resulted in recoveries ranging from 200.97 to 296.12%. The registrant indicated that the elevated recoveries were due to an excess addition of spiking standard to the 0.01 ppm QC control spike sample; these recoveries were not included in the calculation of the mean recovery.

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days) [1]
Olives	≤-10	286-308	Residues of NAA are relatively stable under frozen conditions for up to 365 days.

[1] Refer to the DER for MRID 44835301.

TABLE C.3. Residue Data from Crop Field Trials with NAA.

Trial ID (City, State; Year)	Region	Crop; Variety	Commodity or Matrix	Total Rate (lb ai/A)	PHI (days)	NAA Residues (ppm)
Tulare, CA; 1996 (AA960306.CA1)	10	Olive; Manzanillo	fruit	0.27 + 1.00 = 1.27	112	0.550, 0.578
Kern, CA; 1996 (AA960306.CA2)	10	Olive; Manzanillo	fruit	0.14 + 1.13 = 1.27	102	0.556, 0.610
Tulare, CA; 1996 (AA960306.CA3)	10	Olive; Manzanillo	fruit	1.0 + 0.871 = 1.871	109	0.306, 0.321

TABLE C.4. Summary of Residue Data from Crop Field Trials with NAA.

Commodity	Total Applic. Rate (lb ai/A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT[1]	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
NAA									
Olive	1.27-1.871	102-112	6	0.306	0.610	0.583	0.553	0.487	0.136

[1] HAFT = Highest Average Field Trial.
[2] STMdR = Supervised Trial Median Residue.
[3] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

The submitted olive field trial data indicate that residues of NAA ranged from 0.306 to 0.610 ppm in/on olives harvested 102-112 days following the last of two sequential treatments consisting of: (i) a single spot treatment of the 1.15% RTU formulation (NAA ethyl ester) applied to runoff at a rate of 0.14-1.00 lb ai/A to control suckers and sprouting early in the growing season; and (ii) a single broadcast thinning application of the 24.2% SC formulation (NAA-potassium salt) at 0.871-1.13 lb ai/A.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (11/06/03); Susan V. Hummel (11/06/03)
Petition Number(s): None
DP Barcode(s): D295127
PC Codes: 056003, 056004, 056007, and 056008

Template Version September 2003

NAA and NAA-OEt/PC Codes 056002 and 056008/Amvac Chemical Corporation
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Olive

Primary Evaluator	Gary Otakie, Chemist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03
Reviewer	Susan V. Hummel, Branch Senior Scientist Reregistration Branch 4 Health Effects Division (7509C)	Date: 10/02/03

STUDY REPORT:

44190501 Obrist, J.; Crabtree, K.; Larson, J. (1996) NAA: Olive Metabolism Study Using Naphthaleneacetic Acid and Naphthaleneacetic Acid, Ethyl Ester: Final Report: Lab Project Number: 95458. Unpublished study conducted by ABC Laboratories (Madera, CA) for Amvac Chemical Corporation (Los Angeles, CA). 107 p.

EXECUTIVE SUMMARY:

Amvac Chemical Corporation has submitted an olive metabolism study. A single olive tree was sequentially treated with radiolabeled naphthaleneacetic acid ethyl ester (NAA-OEt) and naphthaleneacetic acid (NAA). Each test substance was radiolabeled on the C-1 position of the naphthalene ring. The NAA-OEt test substance was applied as a 1% ai solution covering about 10% of the tree's new growth tips using a small brush. The NAA test substance was applied using a backpack sprayer as a spray solution at a concentration of 145 ppm ai 62 days following treatment of the same tree with NAA-OEt. Samples of treated olives were harvested at maturity, 118 days following the second application. The harvested olives were rinsed with acetonitrile, pitted, and the olive meat homogenized, extracted with acetonitrile and partitioned into organic and aqueous fractions. Conjugates were released using base hydrolysis. Radioactivity was analyzed by liquid scintillation spectrometry (LSC) with identification by reverse phase HPLC/UV and flow through a radioactivity detector. The total radioactive residues (TRR) were 0.018 ppm in/on whole olive. The major residues found were NAA (8.4%) and conjugates of NAA (55.4%).

There was no NAA-OEt detected in any fraction which was expected due to application of the test substance early in the growing season (without fruit). The remaining extractable radioactivity (55.4% TRR) was characterized to be comprised of five unknowns each present at ≤0.005 ppm. These unknowns were converted to NAA following base hydrolysis of the extracts, and therefore, were characterized as conjugates of NAA. Re-analysis of the organic and aqueous fractions of the extracts at the conclusion of the study demonstrated that residues were stable for the duration of the study.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the olive metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Chapter of the NAA Reregistration Eligibility Decision (RED).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

1-Naphthaleneacetic acid (NAA), its salts, ester, and acetamide are plant growth regulators currently registered for use on various orchard and fruit crops including apple, cherry, olive, orange, pear, tangelo, and tangerine. As plant growth regulators, they may be used on the above-listed crops to prevent preharvest drop of fruits, thin fruit trees, and delay flower induction. They can also stimulate growth and delay leaf drop on ornamentals. The naphthalene acetates are FIFRA List A pesticides assigned to Case No. 0379.

TABLE A.1. Test Compound Nomenclature

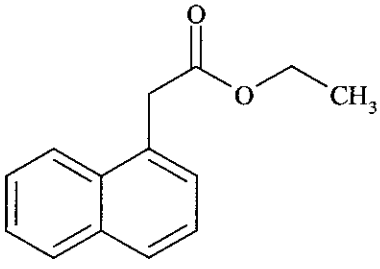
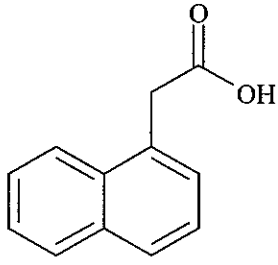
Compound		
Common name	NAA, ethyl ester	NAA
Company experimental name	None	None
IUPAC name	Ethyl 1-naphthaleneacetate	1-Naphthaleneacetic acid
CAS name	1-Naphthaleneacetic acid, ethyl ester	
CAS #	2122-70-5	86-87-3
End-use product/EP	Refer to the Residue Chemistry Chapter of the NAA RED	

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound			
Parameter	NAA-OEt	NAA	
	Value	Value	Reference
Boiling point/range	>150 C	130 C	PC Chapter of the NAA RED
pH	Not available	3.45 (1% slurry; unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Density	1.11 at 20 C	3.75 lb/gal (unspecified temperature)	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Water solubility	Not available	420 mg/L	PC Chapter of the NAA RED
Solvent solubility	soluble in xylene, toluene, ethanol, acetone, and methyl ethyl ketone	freely soluble in acetone, ether, and chloroform.	PC Chapter of the NAA RED
Vapor pressure	Not available	0.3 mm Hg at 26 C	RCB No. 3468 and 3469, 6/3/88, F. Suhre
Dissociation constant, pK_a	Not available	4.3 (unspecified temperature)	RCB No. 3970 and 3971, 7/5/88, F. Suhre
Octanol/water partition coefficient, Log(K_{ow})	Not available	Not applicable; TGAI is very polar	RCB No. 3468 and 3469, 6/3/88, F. Suhre
UV/visible absorption spectrum	Not available	Not available	

B. EXPERIMENTAL DESIGN

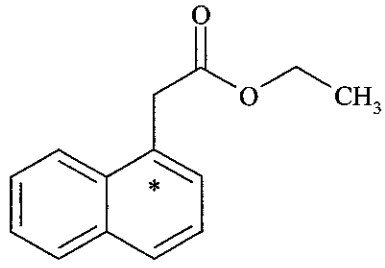
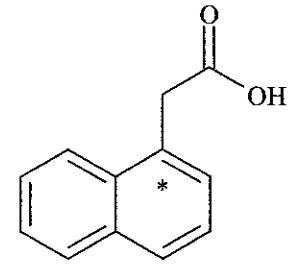
B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information				
Testing Environment	Soil characteristics			
	Type	% OM	pH	CEC (meq/100 g)
Fenced outdoor plots, each containing a single olive tree, at a commercial olive orchard in Madera, CA.	Sandy loam	0.3-0.6	6.9-7.4	5.1-6.9

Daily minimum and maximum temperatures and relative humidity, and precipitation, ground temperatures, and wind speed were reported throughout the study period. Drip irrigation was also provided as needed for normal tree growth. Although historical weather data were not provided, the registrant reported that the recorded climatic conditions were typical for the area.

TABLE B.1.2. Crop Information					
Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Olive/ Miscellaneous Crop	Sevillano	#1 - Early spring on new growth tips #2 - Sixty-two days after first treatment; 12-18 days after full bloom	Mature; 118 days after the last (second) treatment	Olives	Hand-picked

. Test Materials

TABLE B.2.1. Test Material Characteristics		
Chemical structure		
Radiolabel position	1-C position of the naphthalene ring	1-C position of the naphthalene ring
Lot No.	CSL-94-538-49-28	CSL-94-538-51-20
Purity	≥98% (TLC)	≥98% (TLC)
Specific activity	43 mCi/mmol	43 mCi/mmol
Code	[^{14}C]NAA-OEt	[^{14}C]NAA

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information	
Chemical name	[^{14}C]naphthaleneacetic acid, ethyl ester and [^{14}C]naphthaleneacetic acid
Application methods and rates	The NAA-OEt test substance was applied as a 1% ai solution covering about 10% of the tree's new growth tips using a small brush. The NAA test substance was applied using a backpack sprayer as a spray solution at a concentration of 145 ppm ai 62 days following treatment of the same tree with NAA-OEt.
Total Number of Applications Made to the Olive Test Tree	2
Timing of applications	The first application, [^{14}C]NAA-OEt, was applied early spring. The second application, [^{14}C]NAA, was applied 62 days later, 12-18 days after full-bloom.
PHI	118 days

A second olive tree was not treated for control samples.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation

Whole olives were initially rinsed with acetonitrile (ACN). The rinsed fruit were then pitted by hand using a paring knife, and the flesh was weighed and homogenized with dry ice; the pits

were not analyzed. Duplicate samples of homogenized olive flesh were then extracted (2x) with ACN and filtered under vacuum. The filtrates were concentrated and partitioned (3x) with hexane. The hexane phases were combined, but contained no radioactivity and were not further analyzed. The remaining ACN:water phase was diluted with water and partitioned (2x) with dichloromethane (DCM). The aqueous phase was adjusted to pH 1-2 and partitioned (2x) again with DCM. All of the organic phases were combined. The aqueous phase and combined organic phases from the duplicate samples of olives extracted were combined. The organosoluble residues were concentrated for HPLC analysis, and the aqueous-soluble residues were adjusted to pH 5-6, lyophilized, and redissolved in water:methanol (7.5:0.5, v:v) for HPLC analysis.

Subsamples of the aqueous and organic phases were separately subjected to base hydrolysis (refluxing in 10% NaOH for 3.5 hours) for further characterization. The resulting hydrolysates were partitioned with DCM, adjusted to pH 1-2, and then repartitioned with DCM. The acidified DCM fractions were concentrated for HPLC analysis.

B.4.2. Analytical Methodology

Triplicate subsamples of flesh were combusted and radioassayed by liquid scintillation spectrometry (LSC), and the rinsate was radioassayed directly by LSC. The limit of detection (LOD) for the radioassay was 0.001 ppm. Total radioactive residues were calculated by the summation of the radioactivity in the meat and rinsate. The radioactivity in all extracts were determined by LSC, and nonextractable radioactivity was determined by combustion/LSC.

Samples of the rinsate, organic and aqueous phases following DCM partitioning, and acidified DCM fractions of the base hydrolysate were analyzed by reverse-phase HPLC. The RP-HPLC system was equipped with an ODS column, UV (280 nm) and flow-through radioactivity detector. A gradient mobile phase of water and ACN, each with 1% acetic acid, was used. Residues were identified by co-chromatography with unlabeled reference standards of NAA, NAA-OEt, and NAD (1-naphthylacetamide).

The identity of NAA in the organic phase of the olive extract was confirmed using 2D-TLC. TLC analysis was conducted on silica gel 60 F_{254} plates with a solvent system of toluene:acetone:acetic acid (75:25:1, v:v:v) in the first dimension and hexane:ethyl acetate:acetic acid (60:30:1, v:v:v) in the second dimension. Nonlabeled reference standards were co-chromatographed with the sample. Radioactivity was detected by radioanalytical imaging and nonradioactive zones were visualized by UV light.

C. RESULTS AND DISCUSSION

TRR in olives are reported in Table C.2.1. TRR were 0.018 ppm in/on whole olives harvested 118 days following the treatment schedule described. Based on the weight of the olive flesh, the total radioactive residues (TRR) was 0.003 ppm in the acetonitrile rinsate and 0.015 ppm in the

flesh, for a total of 0.018 ppm in/on the whole fruit. Following the acetonitrile rinse, an additional 63.1% of whole fruit TRR was extracted with acetonitrile; the nonextractable residues were 19.2% (0.003 ppm) of the whole fruit TRR. The radioactivity in the acetonitrile extract was partitioned into organic and aqueous fractions. The distribution of the radioactivity in olives is presented in Table C.2.2. The characterization and identification of residues are summarized in Table C.2.3. The majority of the TRR was removed as surface residues (16% TRR) or extracted (84% TRR) from olives using acetonitrile. Extractable residues were partitioned into organic- and aqueous-soluble fractions. Nonextractable residues were 19% of the TRR (0.003 ppm) in olives following solvent extraction.

The free form of the parent, NAA, was the only component identified in olives at 8.4% TRR. The remaining extractable radioactivity was characterized as five unknowns accounting for 55.4% TRR (0.010 ppm); each unknown was present at ≤0.005 ppm. Residues were characterized/identified by HPLC analysis, and NAA was confirmed by TLC analysis. The unknowns were converted to free NAA with base hydrolysis of the organic and aqueous phases of the extract, and therefore characterized as conjugates of NAA.

C.1. Storage Stability

The initial extraction and HPLC analyses of the extracts occurred within 181 days (~6 months) of harvest. The registrant reported that essentially identical results were obtained for the initial and later analyses which demonstrated that residues in the aqueous and organic fractions were stable for the duration of the study.

TABLE C.1. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (C)	Actual Study Duration	Limit of Demonstrated Storage Stability
Olives	-18	181 days (6 months)	Re-analysis of the organic and aqueous extracts 184 days (6.1 months) after the initial analyses.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Olives.

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm)
Olive, rinsate	2	118	0.003
Olive, flesh			0.015
Total			0.018

TABLE C.2.2. Distribution of the Parent and the Metabolites in Olives Following Sequential Applications of [^{14}C]NAA-OEt and [^{14}C]NAA.

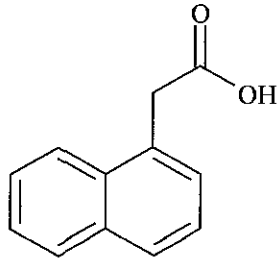
Metabolite Fraction	Whole Olives [1] (TRR = 0.018 ppm)	
	%TRR	ppm
ACN Rinsate	16.1	0.003
NAA	1.5	<0.001
Unknown A	0.6	<0.001
Unknown B1	0.5	<0.001
Unknown B2	11.4	0.002
Unknown C	0.8	<0.001
Uncharacterized	1.3	<0.001
Olive Flesh	83.9	0.015
ACN extract	63.1	0.012
Hexane	None detected	
DCM	25.2	0.005
NAA	5.4	0.001
Unknown A	0.7	<0.001
Unknown B1	10.5	0.002
Unknown B2	3.7	<0.001
Unknown C	3.0	<0.001
Unknown D	0.8	<0.001
Void Volume	0.3	<0.001
Uncharacterized	0.8	<0.001
Aqueous	28.8	0.006
NAA	1.5	<0.001
Unknown A	5.0	0.001
Unknown B1	4.1	<0.001
Unknown B2	13.5	0.003
Unknown C	0.8	<0.001
Void Volume	0.5	<0.001
Uncharacterized	3.4	<0.001
Total extractable	70.1	0.013
Total identified	8.4	0.002
Total unidentified	61.7	0.011
Total bound residues (PES)	19.2	0.003
% Accountability	89.3	

[1] Values are reported as presented by the registrant.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Olives Following Sequential Applications of [^{14}C]NAA-OEt and [^{14}C]NAA.		
Compound	Whole Olives (TRR = 0.018 ppm)	
	%TRR	ppm
Identified:		
NAA	8.4	0.002
Characterized as conjugates of NAA following base hydrolysis of extracts:		
Unknown A	6.3	0.001
Unknown B1	15.1	0.003
Unknown B2	28.6	0.005
Unknown C	4.6	<0.001
Unknown D	0.8	<0.001
Uncharacterized extractable residues (includes void volume)	6.3	0.001
Total identified	8.4	0.002
Total characterized	55.4	0.010
Total extractable	70.1	0.013
Total bound	19.2	0.003

C.3. Proposed Metabolic Profile

No metabolic pathway was presented.

TABLE C.3.1. Identification of Compounds from Olive Metabolism Study		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
NAA	1-Naphthaleneacetic acid	

D. CONCLUSION

The total radioactive residues in/on mature whole olive fruit were 0.018 following sequential applications of [^{14}C]NAA-OEt and [^{14}C]NAA to the same tree at the respective rates described. NAA was the only residue identified and characterized in olives for a total of 63.8% of TRR (8.4% of TRR was free NAA and 55.4% was as conjugates). There was no NAA-OEt detected in any fraction which the registrant believes is not surprising due to the rapid degradation in plants and the fact that NAA-OEt was applied at the beginning of the growing season when the test olive tree did not bear fruits. The Agency concurs with this observation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: Gary Otakie (10/02/03); Susan V. Hummel (10/02/03)
Petition Number(s): None
DP Barcode(s): D232643
PC Codes: 056002 and 056008

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R086913

Chemical:	**1-Naphthaleneacetic acid; 1-Naphthaleneacetamide; Potassium 1-*naphthaleneacetate; Ammonium 1-naphthaleneacetate; Sodium* 1-naphthaleneacetate; Ethyl 1-naphthaleneacetate**
PC Code:	**056002; 056001; 056003; 056004; 056007; 056008**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**11/06/2003**
File ID:	**DPD217162; DPD232643; DPD294864; DPD295126; DPD295127; DPD294853**
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