

**TRADE SECRET**

*Study Title*

**ANALYTICAL ENFORCEMENT PROCEDURE FOR THE ANALYSIS OF  
DPX-KN128/IN-KN127 IN CROPS AND RELATED PROCESS  
FRACTIONS BY GC-MSD**

*Test Guideline*

U.S. EPA Pesticide Assessment Guidelines  
Residue Chemistry - 860 Series, 860.1340 Residue Analytical Method

*Authors of Original Report, Supplement No. 1, Supplement No. 2, and  
Supplement No. 3*

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*Date Study Completed*

Original Report:	June 9, 1995
Supplement No. 1:	February 15, 1996
Supplement No. 2:	March 20, 1997
Supplement No. 3:	November 20, 1997

*Performing Laboratory*

E. I. du Pont de Nemours and Company  
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*Laboratory Project ID*

AMR 3493-95  
Supplement No. 3

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RAG/cja

### **GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

The Good Laboratory Practice (GLP) requirements specified by the EPA in 40 CFR Part 160 and by the Council Directive 91/414/EEC of the Council of the European Communities Concerning the Inclusion of Active Substances in Annex I do not require analytical methods to be developed under Good Laboratory Practices (GLP). However, the methods development presented in this paper was done under GLP except that no protocol was written, no conduct audit was performed, no QA audit of the study records was done, and chromatography data that did not directly support the method as written (e.g., poor recovery data that led to method adjustments) was not retained. Analytical procedures, documentation, and archiving of the validation data were done by Standard Operating Procedures.

- *Sponsor:*

E. I. du Pont de Nemours and Company

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**CERTIFICATION**

**ANALYTICAL ENFORCEMENT PROCEDURE FOR THE ANALYSIS OF  
DPX-KN128/IN-KN127 IN CROPS AND RELATED PROCESS  
FRACTIONS BY GC-MSD**

We, the undersigned, declare that the work described in this supplement was performed under our supervision, and that this report provides an accurate record of the procedures and results.

*Supplement No. 3 by:*

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*Date Original Study Initiated:*

April 18, 1994 (first samples extracted)

*Dates Completed:*

Original Study:	June 9, 1995
Supplement No. 1:	February 15, 1996
Supplement No. 2:	March 20, 1997
Supplement No. 3:	November 20, 1997

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## **ANALYTICAL ENFORCEMENT PROCEDURE FOR THE ANALYSIS OF DPX-KN128/IN-KN127 IN CROPS AND RELATED PROCESS FRACTIONS BY GC-MSD**

*Michael R. Gagnon, Richard A. Guinivan, Paul J. Desmond*

### **REASONS FOR SUPPLEMENT NO. 3**

1) Change 1:

AMR 3493-95, Supplement No. 1, is an analytical procedure for the analysis of DPX-KN128 and IN-KN127 at levels of 0.02 ppm and above in many crops. Supplement No. 1 uses standards prepared in control matrix to eliminate a matrix-induced signal enhancement effect. The matrix effect can be eliminated by dilution and suffice for an enforcement procedure. AMR 3493-95, Supplement No. 2 presents analysis at greater dilutions with standards prepared in organic solvent to allow for enforcement of tolerances at 0.2 ppm or greater (0.3 to 1.0 ppm for some processed fractions). This supplement (Supplement No. 3) reports bridging data to show that samples analyzed by procedures in AMR 3493-95, Supplement No. 1 and AMR 3493-95, Supplement No. 2 give comparable residue values.

2) Change 2:

Change the term 'DPX-KN127' to 'IN-KN127' in the method title to reflect a change in nomenclature.

## **1.0 INTRODUCTION**

DPX-KN128 is the active ingredient in a number of insecticide formulations. An inactive isomer (IN-KN127) is also found in these formulations. The combined isomers can be analyzed in a variety of crops as a single chromatographic peak on non-chiral gas chromatography columns after common extraction and clean-up procedures. Two variations of this analytical approach are given in AMR 3493-95, Supplement No. 1 and in AMR 3493-95, Supplement No. 2. Supplement No. 1 was used for analysis of the magnitude of residue (MOR) studies for watery/non-oily crops. Supplement No. 2 is designed to be an enforcement procedure. The two supplements are summarized below, details can be found in the original write-ups.

AMR 3493-94, Supplement No. 1

The analytes are extracted from 5-g samples into ethyl acetate immediately after the addition of water. An aliquot of ethyl acetate from the single extraction is collected, concentrated under nitrogen, and cleaned up by solid phase extraction with a combination of a silica and a carbon cartridge. The purified sample is then analyzed by capillary gas chromatography with mass selective detection. Validation is done using a variety of crops and some related processing fractions.

Recoveries for fortifications ranging from 0.020-0.25 ppm average 80% to 120% with standard deviations of 4.2 to 18% for a leafy vegetable (lettuce), flowering vegetables (tomato, pepper), cole/brassica (cabbage, broccoli, cauliflower), pome fruit (apples, pears), small fruit (grapes), tomato processing fractions (puree, paste, ketchup, juice), grape processing fractions (juice, wine, raisins, wet pomace, and dry pomace), and apple processing fractions (juice, wet pomace, and sauce) where standards are prepared in control matrix (due to an enhanced signal created by matrix at minimum dilution).

AMR 3493-94, Supplement No. 2

The analytes are extracted from 5-g samples into ethyl acetate immediately after the addition of water. An aliquot of ethyl acetate from the single extraction is collected, concentrated under nitrogen, and cleaned up by solid phase extraction with a combination of a silica and a carbon cartridge. The purified sample is then analyzed in ethyl acetate by capillary gas chromatography with mass selective detection with standards prepared in ethyl acetate. Validation is done using a variety of crops and some related processing fractions.

Practical quantitation levels were 0.2 ppm and above for lettuce, tomato, pepper, cabbage, broccoli, cauliflower, apples, pears, and applesauce; and 0.3 ppm and above for ketchup and apple wet pomace, and 1.0 ppm and above for tomato paste.

Recoveries for fortifications which range from 0.20-1.5 ppm average 86-103% with standard deviations of 7.6 to 16%.

The control chromatograms have no peaks and generally the fortified sample chromatograms contain only the analyte peak.

This method specifies that m/e 527 be monitored. This is appropriate if controls are available. If analyses are conducted on samples where no controls are available, multiple ions can be monitored for confirmation if initial analysis with ion 527 indicates that DPX-KN128/IN-KN127 is present. Standards as low as 0.020 µg/mL and samples as low as 0.20 ppm have shown good ion ratio agreement for m/e 218, 321, and 527.

## 2.0 EXPERIMENTAL

Residue data was generated for this Supplement 3 from 5 samples from each of 4 crops (tomatoes, apples, lettuce, and broccoli). These samples were chosen from magnitude of residue studies and had confirmed incurred residues. The samples with the highest level residues at the maximum recommended label rate were chosen from each crop as most likely to represent tolerance levels.

Procedures in both AMR 3493-95, Supplement No. 1 (MOR analysis method) and Supplement No. 2 (proposed enforcement method) were done for each sample. Supplement No. 1 quantitates at a limit of quantitation of 0.02 ppm with minimum dilution and standards prepared in control matrix to accommodate a matrix induced chromatographic enhancement. Supplement No. 2 calls for greater dilution to eliminate the matrix enhancement and allow for standards in ethyl acetate. Since some of the sample residues were quite high (e.g. lettuce at 12 ppm) the samples were diluted above levels specified in Supplement No. 2 when analyzed by Supplement No. 1 procedures in MOR studies, however, they were still quantitated against standards in control matrix. Therefore, initial Supplement No. 1 and Supplement No. 2 analyses were done on the same sets of fortifications and treated samples at identical dilutions appropriate for the incurred residue levels.

Chromatographic response against standards in ethyl acetate (Supplement No. 2) is lower than response against standards in control matrix (Supplement No. 1). Initial Supplement No. 2 analyses were found to have low chromatographic signal-to-noise for the low fortifications and lower level treated samples. The residue values appeared to be inflated due to inclusion of chromatographic baseline noise during integration for apple, broccoli, and tomato samples at lower level residues and fortifications. Lettuce samples were not affected because the residues were so high that there was significant signal-to-noise for all fortifications and samples at the dilutions used. Therefore, apple, broccoli, and tomato samples were concentrated to half their initial volume and reanalyzed at a greater signal-to-noise ratio.

## 3.0 RESULTS

Table 1 is a compilation of the recovery data generated from fortifications run with all the analytical sets. Overall recovery for the 4 crops is  $93 \pm 18\%$  (relative standard deviation of 20%) for 16 fortifications.

Residue values combined for all 4 crops averaged 2.8 ppm (standard deviation of  $\pm 4.3$ ,  $N = 20$ ) for samples analyzed by AMR 3493-95, Supplement No. 1. Residue values combined for all 4 crops averaged 2.5 ppm (standard deviation of  $\pm 3.6$ ,  $N = 20$ ) for samples analyzed by AMR 3493-95, Supplement No. 2.

#### 4.0 CONCLUSION

The procedures in both AMR 3493-95, Supplement No. 1 (MOR analysis method), and AMR 3493-95, Supplement No. 2 (proposed enforcement method) give similar residue values for lettuce, apple, broccoli, and tomato analyses.

#### 5.0 REFERENCES

1. Gagnon, M.R., Guinivan, R.A. "Residue Procedure for the Analysis of DPX-KN128/DPX-KN127 in Crops and Related Process Fractions by GC-MSD", DuPont Report No. AMR 3493-95, 1997; Supplement No. 1, DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
2. Gagnon, M.R., Guinivan, R.A., Desmond P.J. "Analytical Enforcement Procedure for the Analysis of DPX-KN128/DPX-KN127 in Crops and Related Process Fractions by GC-MSD", DuPont Report No. AMR 3493-95, Supplement No. 2, 1997; DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
3. Behmke, F.D., Klemens, F.K. "Magnitude and Decline of Residues of DPX-KN128 and IN-KN127 in Lettuce Following Application of DPX-MP062 and DPX-JW062 Experimental Insecticides at Maximum Label Rates", DuPont Report No. AMR 3728-96, 1997; DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
4. Gagnon, M.R., Klemens, F.K. "Magnitude and Decline of Residues of DPX-KN128 and IN-KN127 in Apples Following Application of DPX-MP062 and DPX-JW062 Experimental Insecticides at Maximum Label Rates", DuPont Report No. AMR 3950-96, 1997; DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
5. McVicker, J.D., Klemens, F.K. "Magnitude and Decline of Residues of DPX-KN128 and IN-KN127 in Broccoli Following Application of DPX-MP062 and DPX-JW062 Experimental Insecticides at Maximum Label Rates", DuPont Report No. AMR 3732-96, 1997; DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
6. Adams, G.M., Klemens, F.K. "Magnitude and Decline of Residues of DPX-KN128 and IN-KN127 in Tomatoes Following Application of DPX-MP062 and DPX-JW062 Experimental Insecticides at Maximum Label Rates", DuPont Report No. AMR 3733-96, 1997; DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.

**TABLE 1**  
**RECOVERY OF DPX-KN128/IN-KN127 FORTIFICATIONS<sup>a</sup> FROM SAMPLE**  
**SETS ANALYZED TO PRODUCE RESIDUE DATA**

SAMPLE	STANDARDS PREPARED IN:	PPM ADDED	PPM FOUND	% RECOVERY	
Lettuce	control matrix	6.5	5.5	85	Avg. = 92 <sup>e</sup>
	control matrix	10.5	10	100	S.D. <sup>b</sup> = ± 9.6
	ethyl acetate	6.5	6.5	100	R.S.D. <sup>c</sup> = 10
	ethyl acetate	10.5	8.6	82	N = 4
Apples	control matrix	0.75	0.67	89	Avg. = 86 <sup>e</sup>
	control matrix	1.5	1.3	88	S.D. <sup>b</sup> = ± 13
	ethyl acetate	0.75	0.99	132, 65 <sup>d</sup>	R.S.D. <sup>c</sup> = 15
	ethyl acetate	1.5	1.1	76, 62	N = 4
Broccoli	control matrix	0.4	0.35	87	Avg. = 85 <sup>e</sup>
	control matrix	1.0	0.87	87	S.D. <sup>b</sup> = ± 13
	ethyl acetate	0.4	0.53	132, 65 <sup>d</sup>	R.S.D. <sup>c</sup> = 16
	ethyl acetate	1.0	0.78	78, 55	N = 4
Tomato	control matrix	0.10	0.081	81	Avg. = 82 <sup>e</sup>
	control matrix	0.25	0.22	86	S.D. <sup>b</sup> = ± 10
	ethyl acetate	0.10	0.11	113, 70 <sup>d</sup>	R.S.D. <sup>c</sup> = 12
	ethyl acetate	0.25	0.18	72, 64	N = 4
				OVERALL	Avg. = 86 <sup>e</sup>
					S.D. <sup>b</sup> = ± 11
					R.S.D. <sup>c</sup> = 13
					N = 16

<sup>a</sup> The ppm found is rounded to 2 significant figures while % Recovery is rounded to the nearest whole percent. The calculations are done on a computer spreadsheet with all decimal places carried through the computations. Values presented are taken from the spreadsheet and rounded as specified. Therefore, % Recovery reported may be slightly different than a division of ppm found by ppm added in this table.

<sup>b</sup> Standard Deviation.

<sup>c</sup> Relative Standard Deviation.

<sup>d</sup> Initial result probably inflated due to quantitation at low signal-to-noise levels (see discussion in Section 2.0).

<sup>e</sup> Averages were calculated using the mean value for samples with duplicate injections.

**TABLE 2**  
**DPX-KN128/IN-KN127 RESIDUES FOUND IN MAGNITUDE OF RESIDUE**  
**(MOR) SAMPLES ANALYZED BY BOTH AMR 3493-95, SUPPLEMENT NO. 1**  
**AND AMR 3493-95, SUPPLEMENT NO. 2, PROCEDURES**

CROP <sup>d</sup>	SAMPLE NO. (S00...)	PPM FOUND <sup>a, c</sup>		PPM FOUND <sup>b, c, e</sup>
		SUPPLEMENT NO. 1	SUPPLEMENT NO. 2	
lettuce	122465	6.5	9.0	Avg. = 9.8
"	210311	9.8	9.9	Supp. 1
"	210312	8.6	9.0	Avg. = 8.5
"	210317	12	7.2	Supp. 2
"	210318	12	7.2	
apples	185519	0.27	0.26, 0.24	Avg. = 0.67
"	186060	0.86	0.92, 0.76	Supp. 1
"	186080	0.86	1.0, 0.87	Avg. = 0.66 <sup>f</sup>
"	225129	0.58	0.57, 0.50	Supp. 2
"	225138	0.80	0.82, 0.65	
broccoli	072639	0.73	1.4, 0.70	Avg. = 0.44
"	072644	0.49	0.74, 0.58	Supp. 1
"	194143	0.32	0.38, 0.37	Avg. = 0.59 <sup>f</sup>
"	194149	0.29	0.35, 0.34	Supp. 2
"	194150	0.39	0.47, 0.45	
tomato	123711	0.14	0.22, 0.18	Avg. = 0.11
"	123717	0.13	0.22, 0.19	Supp. 1
"	123718	0.13	0.19, 0.20	Avg. = 0.20
"	176984	0.20	0.28, 0.27	Supp. 2
"	176991	0.086	0.12, 0.095	
	Overall Avg.		2.8	2.5 <sup>f</sup>
	Overall SD		±4.3	±3.6
	N		20	20

<sup>a</sup> Standards prepared in control matrix.

<sup>b</sup> Standards prepared in ethyl acetate.

<sup>c</sup> Data reported to 2 significant figures.

<sup>d</sup> See reference list for the crop magnitude of residue studies from which these samples were selected.

<sup>e</sup> Initial results for some apple, broccoli, and tomato samples were probably inflated due to quantitation at low signal-to-noise levels; therefore, a second analysis was done after a 2 mL to 1 mL concentration (see discussion in Section 2.0).

<sup>f</sup> Averages were calculated using the mean value for all samples with duplicate injections.