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4-11-95

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

APR 11 1995

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Vinclozolin.** Consumer Practice (Washing) Study Reregistration Case No. 2740 Chemical No. 113201 MRID #42305600 and 42305601 DP Barcode D213432 CBRS #15295

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A number of tolerances for vinclozolin and its metabolites containing the 3,5-dichloroaniline moiety have been established, including 25 ppm on stone fruit, 10 ppm on strawberries, raspberries, lettuce and kiwi fruit, 6 ppm on grapes, 5 ppm on endive, 3 ppm on peppers and tomatoes and 1 ppm on onions (dry bulb) in 40 CFR 180.380. Food Additive tolerances have been established at 75 ppm on prunes and 30 ppm on raisins in 40 CFR 185.1850 and a Feed Additive tolerance of 42 ppm has been established on dry grape pomace in 40 CFR 186.1850. Several petitions are under review.

The latest DRES analysis for vinclozolin (memo from J. Wintersteen to H. Jamerson dated 3/20/95) indicates that for acute effects, the 90th percentile MOE is 63 for the most highly exposed group (females over the age of 13). Concerning chronic effects, for the US population, 31% of the R_fD is consumed and for children 1-6, 64% of the R_fD is consumed.

BASF, the registrant, has submitted a consumer practice study (MRID #42305601). This study demonstrates the effects of washing and washing/towel drying on surface applied residues of vinclozolin for peaches, nectarines, and leaf lettuce (vinclozolin applied 2 or 24 hours before washing). These data are reviewed below.

Recommendations

The submitted study indicates that when vinclozolin was applied to the surface of peaches, nectarines, and leaf lettuce and allowed to dry for 24 hours, the resulting residues in/on the commodities can be reduced by washing and/or washing/towel drying the commodities.

Metabolism studies indicate that vinclozolin and its metabolites containing the 3,5-dichloroaniline moiety are not just found on the surface of commodities following commercial application practices (and associated PHIs), but instead are translocated into the commodities, followed by metabolism/conjugation. Therefore, the value of this consumer practice study for revising exposure estimates is minimal.

Background

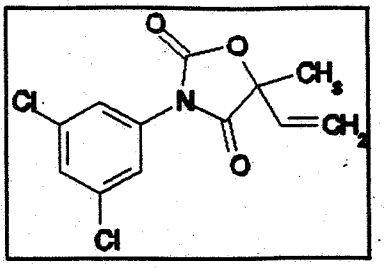
A CBRS (then RCB) memo dated 1/18/80 (R. Davis to H. Jacoby), contained a review of a lettuce metabolism study submitted in conjunction with PP#9G2204. When uniformly phenyl labeled ^{14}C-vinclozolin was applied to lettuce at the two true leaf stage and 19 days later at 1.0 lb ai/A/application, autoradiography indicated uniform translocation within one day from the foliar application site throughout the plant. This memo also contained a review of a peach metabolism study. In that study, peaches were painted evenly with a 0.1% aqueous solution of uniformly phenyl labeled ^{14}C-vinclozolin. The stone from one peach in this study was analyzed (nut meat and shell were analyzed separately). A ^{14}C-vinclozolin metabolite was detected in the shell (this metabolite had not been identified in earlier peach metabolism studies). These studies indicate that vinclozolin is taken up into peaches and lettuce, and does not remain only on the surfaces on these commodities.

CBRS also notes that other submitted metabolism studies indicate significant conjugation of vinclozolin metabolites. G.Makhinijani (memo dated 1/19/79, PP#8G2068) noted that in strawberries, "... metabolites B and T represented the main portion (38%) of metabolized material but occur only in conjugated form." R. Davis (1/18/80, PP#9G2204) also noted that the major metabolites formed in lettuce were B, E, and S and that Metabolite B was conjugated to pectin. See Figure 1 (page 3) for structures of vinclozolin and its plant metabolites. CBRS notes that these metabolites are determined by the analytical method.

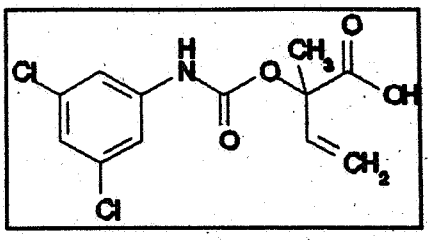
CBRS believes that the fact that vinclozolin metabolites are found conjugated also supports the contention that vinclozolin residues are not found solely on the surfaces of commodities.

Figure 1. Vinclozolin and plant metabolites.

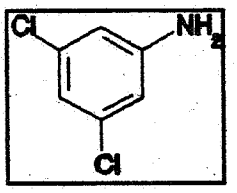
a) **Vinclozolin**



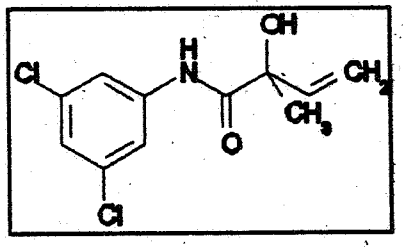
b) 3-(3,5-dichlorophenyl)carbamic acid (1-carboxy-1-methyl)allyl ester (**Metabolite B**);



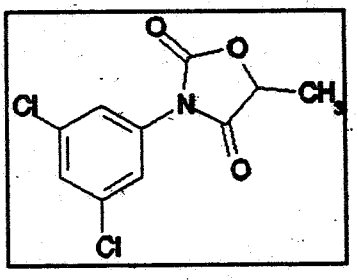
c) 3,5-dichloroaniline (**Metabolite D**);



d) N-(3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide (**Metabolite E**);



e) 3-(3,5-dichlorophenyl)-5-methyl-1,3-oxazolidine-2,4-dione (**Metabolite S**)



Conclusions

1. The procedure used for applying vinclozolin (Ronilan DF and Ronilan WP formulations were used) to commodities in the study did not adequately simulate commercial application. The PHIs for stone fruit and lettuce (14 and 21 days respectively are much longer than the treatment to washing intervals used in the study (24 hours). As indicated by the peach and lettuce metabolism studies, vinclozolin apparently is translocated into commodities, where it is metabolized and conjugated.

2. The washing and washing/towel drying procedures used on treated samples were adequately described.

3. The analytical method used was adequately described. The method validation results provided indicate that the method is acceptable for analysis of vinclozolin surface residues on stone peaches, nectarines, and lettuce.

4. Peaches - Results for peaches which were washed and washed/towel dried are summarized in Tables 2, 3 and 6. For peaches fortified at 25 ppm, residues in washed peaches were higher 24 hours following application of vinclozolin (average 8.4 ppm) versus residues observed upon washing peaches 2 hours after vinclozolin application (average 5.7 ppm). For washed and towel dried peaches, this difference was not as great (24 hours post application, average 5.4 ppm; and 2 hours post application average 5.3 ppm). These data indicates that vinclozolin residues are translocated into the peach over time. As might be expected, washing and towel drying removed more residues than washing alone. Additionally, more residues remained after washing or washing/towel drying when the WP formulation was used for vinclozolin application versus the DF formulation.

5. Nectarines - Results for nectarines which were washed and washed/towel dried are summarized in Tables 4 and 6. As was the case for peaches, vinclozolin residues in nectarines washed and analyzed 24 hours after vinclozolin application were higher than those obtained in nectarines washed and analyzed 2 hours after vinclozolin application. For nectarines washed 2 hours post application the average residue was 2.3 ppm. For nectarines washed 24 hours post application the average residue was 3.3 ppm. For washed/towel dried nectarines, 2 hours post application, average residue was 2.4 ppm and for washing/towel drying 24 hours post application average residues were 2.7 ppm. These data indicates that vinclozolin residues are translocated into the nectarine over time. A greater percentage of residues were removed by washing and washing/drying of nectarines versus peaches. More residues remained after washing or washing/towel drying when the WP formulation was used for vinclozolin application.

6. Lettuce - Results for lettuce leaves which were washed and washed/towel dried are summarized in Tables 5 and 6. The formulation applied did not appear to make a difference on the amount of vinclozolin remaining. Also, the amount remaining did not appear to be different for washed and washed/towel dried lettuce. All lettuce samples were analyzed 2

hours after application of vinclozolin.

Detailed Considerations

The study sponsor was BASF Corporation and the performing laboratory was EN-CAS Analytical Laboratories, Winston-Salem, NC. The study was entitled "Vinclozolin Consumer Practice Study, March 30, 1992, BASF Study #91053".

The purpose of the study was to determine the effect of washing and washing/towel drying practices on the removal of surface applied residues of vinclozolin from stone fruit and leaf lettuce (residues applied at 2 or 24 hours before washing).

Label Directions for Vinclozolin Use on Stone Fruit and Lettuce - Stone fruit label directions for Ronilan Fungicide state that for control of brown rot blossom and twig blight, applications may be made at early bloom, full bloom, and at petal fall. Applications may be made at 0.5 to 1.0 lb ai/A/application. Do not apply more than 3 bloom treatments. To control fruit brown rot, apply at up to 1.0 lb ai/A when conditions favor disease development within 3 weeks of harvest. Do not apply more than 4 lb ai/A/season (3 lb ai/A at bloom and 1 lb ai/A pre-harvest). Do not apply more than one pre-harvest treatment. Do not apply within 14 days of harvest (14 day PHI). Application rates are based on an application of 400 gallons per acre of dilute spray.

Lettuce label directions permit up to three applications per season, at rates up to 1.0 lb ai/A/application. Do not apply more than 3.0 lb ai/A/season. There is a 21 day PHI. Applications should be made in not less than 100 gallons of spray solution per acre.

Samples - Freestone peaches, nectarines, and lettuce used in the study were obtained from local grocers in the Winston-Salem NC area from 7/25/91 to 10/16/91. Because of problems with background interferences in the store bought peaches, control peaches were obtained from BASF on 9/17/91. Samples were stored chilled in a refrigerator (1 C to 3 C) until they were used in the study. The maximum time that fruits were stored between receipt and use was 21 days. Lettuce was used with 4 days of receipt.

Application - Ronilan DF (48.7% ai) and Ronilan WP (49.5% ai) supplied by BASF were used in the study. Lot number, expiration date, and appearance were reported. The 3,5-DCA reference standard (98%) was supplied by Chem Service (West Chester, PA). All test and reference substances and standard solutions were stored at -10 to -17 C. Stock solutions of the DF and WP were prepared using Milli-Q water. The 3,5-DCA stock solution was prepared in hexane.

Peaches - The analyte was applied to each peach at room temperature to provide a nominal vinclozolin fortification level of either 5 or 25 ppm (ug/g) based on a nominal weight of 150 g for an average size peach. Therefore, quantities of Ronilan suspensions containing either 791 ug (5 ppm) or 4260 ug (25 ppm) of vinclozolin were applied to the surface of each

peach. This quantity was divided by the actual weight of the peach to determine the actual fortification level for the individual peaches. To perform each application, each peach was weighed then clamped to a ring stand with a three point clamp. Between 40 to 60 uL of a continuously stirred suspension containing either 4058 ug/mL or 14287 ug/mL of vinclozolin was applied. Using a micropipet, a circle of eight droplets of approximately 5 uL each, were applied to the surface of the peach, evenly spaced around the equator of each peach. A similar second concentric circle of drops was applied about one third the distance between the equator and top apex of the peach. A similar third concentric circle of drops was applied about two thirds the distance between the equator and top apex of the peach. The peaches were then slowly inverted by rotating the attached clamps. The applied droplets were sufficiently small that they did not run when the fruit was rotated. Two additional concentric circles of drops were then applied to the unfortified half of the peach. Following application, the peaches were allowed to dry for 2 or 24 hours as required by the experimental procedure.

Unwashed Peaches - Following the drying time, each peach was cut in half in order to remove the pit and the peach meat was sliced into thin wedges and placed into a sample flask for analysis. Care was taken during slicing to avoid dislodging residues (peaches were handled at points that did not contain droplets of vinclozolin).

Washed Peaches - Treated peaches were washed under cold tap water at a calibrated flow rate of 5000 mL/min. During washing, the analyst wore polypropylene gloves and applied light rubbing pressure to the peaches while they were under the tap water. the peaches were constantly turned and lightly rubbed during the time 5 second wash interval. Washed peaches were then cut up and analyzed.

Washed and Dried Peaches - Each peach was fortified and washed as described above, then subjected to towel drying by thoroughly drying it with a clean cotton cloth, using gentle pressure. Washed and dried peaches were then cut up and analyzed.

Nectarines - All fortifications and washing, washing/drying procedures were just as for peaches, except that nectarines were only fortified at 25 ppm.

Leaf Lettuce - Lettuce leaves approximately 4 to 6 inches wide were used in this study. Quantities of **Ronilan** (WP or DF) suspension were applied to each lettuce leaf at a nominal vinclozolin fortification level of 10 ppm based on a nominal weight of 50 g for an average sized lettuce leaf (495 to 499 ug of vinclozolin/leaf). The quantity of vinclozolin applied was divided by the actual weight of the lettuce leaf to accurately determine the actual amount applied. For application, weighed leaves were placed on paper towels. 40 ul aliquots of a continuously stirred suspension containing either 2435 ug/mL or 2475 ug/mL of vinclozolin were applied. A total of 200 to 205 uL (495 ug to 499 ug) was applied to each lettuce leaf sample. For each sample, 5 uL drops were applied randomly, but evenly spaced on the upright surface of the leaf. The drops were allowed to dry for at least 2 hours at ambient temperature prior to analysis.

Unwashed - Following drying, the fortified surface of each lettuce sample was rolled inward and the leaf was placed into a sample flask for analysis.

Washed - Treated lettuce leaves were washed under cold tap water at a calibrated flow rate of 5000 mL/min. During washing, the analyst wore polypropylene gloves and applied light rubbing pressure to the leaves while they were under the tap water. Samples were constantly turned and lightly rubbed during the time 5 second wash interval. Washed samples were then placed into sample flasks for analysis.

Washed and Dried Peaches - Each lettuce leaf was fortified and washed as described above, then subjected to towel drying by thoroughly drying it with a clean cotton cloth, using gentle pressure. Washed and dried samples were then placed into a sample flask for analysis.

Analytical Method - the analytical method used in the study was EN-CAS Method Number ENC-10/91, entitled "Analytical Method for the Determination of Vinclozolin as 3,5-Dichloroaniline (3,5-DCA) in Peaches, Nectarines, and Lettuce", dated 1/14/92. Briefly, samples were placed in a flat bottom boiling flask and then hydrolyzed with NaOH (500 mL of 5 N NaOH) converting vinclozolin to 3,5-DCA. The hydrolysis product was steam distilled and simultaneously partitioned into hexane using a Nielsen-Kryger distillation apparatus. All matrices required a minimum of 6 hours for steam distillation. After distillation, the hexane and aqueous fractions were frozen to facilitate separation of hexane and aqueous layers. After freezing, the hexane layer was removed and either dried over anhydrous sodium sulfate and diluted for direct GC analysis of 3,5-DCA (for peaches and nectarines) or further cleaned up using silica gel (lettuce). GC analyses were performed using a fused silica capillary 30 M x 0.32 mm , 0.25 um film thickness DB-17 column and alkali flame (nitrogen/phosphorus) detection. Method validation results are reported below.

Sample residues were corrected for procedural recoveries. Procedural recovery samples were fortifications made into flasks containing ground fruit or chopped lettuce samples.

Adequate representative chromatograms were provided for standards and for control and fortified samples. A representative standard curve was also provided.

Results

Method Validation - Method validation was performed for both Ronilan formulations on each matrix. Results are reported in Table 1. FOr peaches recoveries ranged from 73% to 98%, for nectarines 85% to 109%, and for lettuce 82% to 100%.

The range of recoveries obtained in the method validation are within Agency guidelines. CBRS concludes that the method is adequate for analysis of vinclozolin in/on peaches, nectarines, and lettuce.

Table 1. Results of method validation study. Samples were fortified with either Ronilan WP or DF formulations. Reported results reflect subtraction of average levels found in controls.

Sample	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)	% Recovery
Peaches	Controls	–	0.044	–
			<0.10	–
	WP	0.11	0.081	73
		0.10	0.080	80
		33	29.3	89
		26	22.3	86
	DF	0.11	0.095	87
		0.10	0.098	98
		31	30.4	98
		26	21.1	81
Nectarines	Control	–	0.038	–
			0.058	–
	WP	0.10	0.102	102
		30.0	27.0	90
	DF	0.10	0.085	85
		29.7	32.6	109
Lettuce	Control	–	0.094	–
			0.090	–
	WP	0.10	0.082	82
		15	13.8	92
	DF	0.10	0.083	83
		15	14.96	100

Procedural recovery sample data were also provided (6 datasets for peaches, 3 for nectarines, and 3 for lettuce). For peaches recoveries ranged from 45% to 136% (fortifications at 0.1 to 25 ppm, n = 33), for nectarines 66% to 137% (fortifications at 0.1 and 25 ppm, n = 12), and for lettuce 75% to 134% (fortifications at 0.1 and 10 ppm, n = 12). For peaches fortified at 5 ppm, recoveries were 87%$\pm$13% (n = 6) and for fortifications at 25 ppm recoveries were 94%$\pm$23% (n = 6). For nectarines fortified at 25 ppm, procedural recoveries averaged 98%$\pm$23% (n = 6), and for lettuce fortified at 10 ppm, recoveries were 100%$\pm$19% (n = 6).

Peaches - Results for peaches which were washed and washed/towel dried are summarized in Tables 2, 3 and 6. For peaches fortified at 25 ppm, residues in washed peaches were higher 24 hours following application of vinclozolin (average 8.4 ppm) versus residues observed upon washing peaches 2 hours after vinclozolin application (average 5.7 ppm). For washed and towel dried peaches, this difference was not as great (24 hours post application, average 5.4 ppm; and 2 hours post application average 5.3 ppm). These data indicate that vinclozolin residues are translocated into the peach over time. As might be expected, washing and towel drying removed more residues than washing alone. Additionally, more residues remained after washing or washing/towel drying when the WP formulation was used for vinclozolin application.

Table 2. Results of consumer practices on vinclozolin residues in/on peaches fortified with vinclozolin at 25 ppm.

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
None (unwashed)	2	WP	24.9	27.0	--
			25.1	34.8	--
			24.8	30.9	--
	24	WP	25.0	33.5	--
			25.1	34.1	--
			24.8	31.5	--
	24	DF	24.7	25.2	--
			24.9	22.6	
			24.7	22.4	
Washed	2	WP	25.0	4.69	19
			25.2	4.95	20
			25.0	7.50	30
	24	WP	25.1	7.01	28
			25.0	7.99	32
			25.1	10.3	41
	24	DF	24.9	10.3	41
			25.0	4.43	18
			25.1	2.67	11

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
Washed and Towel Dried	2	WP	25.3	4.46	18
			24.9	6.20	25
			25.2	5.86	23
	24	WP	24.9	4.78	19
			25.2	5.71	23
			25.1	5.81	23
	24	DF	25.0	2.55	10
			25.2	3.21	13
			25.1	2.53	10

Table 3. Results of consumer practices on vinclozolin residues on peaches fortified with vinclozolin at 5 ppm.

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
None (unwashed)	24	WP	5.03	4.83	–
			5.01	3.89	–
			4.99	4.21	–
	24	DF	5.01	5.43	–
			5.01	5.04	–
			5.01	5.67	–
Washed	24	WP	5.01	1.65	33
			5.02	2.51	50
			5.01	1.16	23
	24	DF	24.9	0.63	13
			25.0	0.59	12
			25.1	0.50	10

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
Washed and Towel Dried	24	WP	5.03	0.94	19
			5.01	0.86	17
			5.02	1.48	29
	24	DF	5.02	0.30	6
			5.04	0.39	8
			5.02	0.32	6

Nectarines - Results for nectarines which were washed and washed/towel dried are summarized in Tables 4 and 6. As was the case for peaches, vinclozolin residues in nectarines washed and analyzed 24 hours after vinclozolin application were higher than those obtained in nectarines washed and analyzed 2 hours after vinclozolin application. For nectarines washed 2 hours post application the average residue was 2.3 ppm. For nectarines washed 24 hours post application the average residue was 3.3 ppm. For washed/towel dried nectarines, 2 hours post application, average residue was 2.4 ppm and for washing/towel drying 24 hours post application average residues were 2.7 ppm). These data indicate that vinclozolin residues are translocated into the nectarine over time. A greater percentage of residues were removed by washing and washing/drying of nectarines versus peaches. More residues remained after washing or washing/towel drying when the WP formulation was used for vinclozolin application.

Table 4. Results of consumer practices on vinclozolin residues in/on nectarines.

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
None (unwashed)	2	WP	25.0	18.6	--
			25.0	18.2	--
			25.1	17.4	--
	24	WP	25.1	18.5	--
			25.1	13.3	--
			24.9	16.8	--
	24	DF	25.0	23.3	--
			25.0	22.6	--
			25.1	23.1	--

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
Washed	2	WP	25.2	2.13	8
			25.1	3.38	13
			25.0	1.49	6
	24	WP	25.0	2.97	12
			25.0	3.58	14
			25.1	3.30	13
	24	DF	25.1	2.67	11
			25.0	1.91	8
			25.2	2.26	9
Washed and Towel Dried	2	WP	25.1	3.40	14
			25.0	1.68	7
			25.0	1.99	8
	24	WP	25.1	2.65	11
			24.9	2.03	8
			25.1	3.48	14
	24	DF	25.1	1.63	6
			25.0	2.88	12
			25.0	1.92	8

Lettuce - Results for lettuce leaves which were washed and washed/towel dried are summarized in Tables 5 and 6. The formulation applied did not appear to make a difference on the amount of vinclozolin remaining. Also, the amount remaining did not appear to be different for washed and washed/towel dried lettuce. All lettuce samples were analyzed 2 hours after application of vinclozolin.

Table 5. Results of consumer practices on vinclozolin residues in/on lettuce.

Practice	Drying Time (hr)	Formulation	Fortification Level (ppm)	Vinclozolin Found (ppm)[a]	% Vinclozolin Remaining After Practice
None (unwashed)	2	WP	9.9	8.15	--
			9.9	9.89	--
			9.9	9.18	--
	2	DF	10	12.9	--
			10	11.2	--
			10	10.4	--
Washed	2	WP	9.9	2.87	29
			9.9	2.87	29
			9.9	2.78	28
	2	DF	10	3.70	37
			10	2.24	22
			10	2.22	22
Washed and Towel Dried	2	WP	9.9	3.10	31
			9.9	3.29	33
			9.9	3.07	31
	2	DF	10	1.43	14
			10	3.70	37
			10	2.50	25

Table 6. Summary of results for consumer practice study. Only results for 24 hour drying times for peaches and nectarines were considered. Results for different formulations used on all samples were combined.

Sample	Nominal Fortification Level (ppm)	Consumer Practice	Average % Vinclozolin Remaining ± SD	Number of samples	Range (%)
Peaches	5	Washing	24% ± 16%	6	10 - 50
		Washing/Drying	14% ± 9%	6	6 - 29
	25	Washing	29% ± 12%	6	11 - 41
		Washing/Drying	16% ± 6%	6	10 - 23
Nectarines	25	Washing	11% ± 2%	6	8 - 14
		Washing/Drying	10% ± 3%	6	6 - 14
Lettuce	10	Washing	28% ± 6%	6	22 - 37
		Washing/Drying	29% ± 8%	6	14 - 37

cc: S.F., circ., R.F., List B File, S.Knizner
RDI: A. Rathman, 4/6/95 M.Metzger, 4/7/95 F.Suhre, 4/7/95
7509C:CBRS:CM#2:305-6903:SAK:sak:Vinclo:4/5/95