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Determination of Fungicide Residues in Field-Grown Strawberries following Different Fungicide Strategies against Gray Mold (*Botrytis cinerea*)

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In a 2 year experiment, residues in field-grown strawberries were investigated from the fungicides fenhexamid, pyrimethanil, tolylfluanid, and kresoxim-methyl resulting from different strategies, as regards the dose, number, and time of fungicide applications. Kresoxim-methyl was only used the first year and in full or no dose to control powdery mildew. In the first year, the highest concentrations analyzed were 0.66 mg kg^{-1} for pyrimethanil and 0.63 mg kg^{-1} for fenhexamid resulting from the use of recommended dose rates and a preharvest interval (PHI) of 10 days, thus not exceeding the Danish maximum residue limit (MRL) of 1 mg kg^{-1}. Tolylfluanid was used no later than 21 days before harvest, which left residue contents in the berries of 0.48 mg kg^{-1}, a value well below the MRL of 5 mg kg^{-1}. In the second year, fungicide residues found in the samples were generally lower, 0.39 and 0.03 mg kg^{-1} for pyrimethanil and fenhexamid, respectively. No residues of kresoxim-methyl were found in any of the samples from the field trials, indicating that kresoxim-methyl residues had declined to a level well below the detection limit within the 28 day period between the last application and the harvest.

KEYWORDS: DMST; fenhexamid; *Fragaria* × *ananassa*; fungicides; kresoxim-methyl; LC-MS; pyrimethanil; residues; strawberry; tolylfluanid

INTRODUCTION

Consumer awareness and concern for pesticide residues in food products is challenging fruit and vegetable growers to minimize pesticide residues without increasing production risks. Results reported from the European Commision of pesticide residues in Danish strawberries (*Fragaria* × *ananassa*) in the years 1996−2000 showed that approximately half of the strawberry samples tested contained fungicide residues (*1*). Residues did not exceed the maximum residue limits (MRLs) in any case, but even smaller amounts may attract quite some attention in consumer's conception of food safety. Evaluation and communication of risk as demonstrated by Harris et al. (*2*) and Low et al. (*3*) are indeed a future challenge for advisors, researchers, members of official authorities, and other people working in different areas of the food industry. For growers of fruit and vegetables, it is important to focus on continuous progressive improvement of their management and production practices to avoid bad publicity and to support continued consumer confidence. A descriptive analysis of the differences in pesticide residues resulting from different production practices was made by Baker et al. (*4*). One way of improving production is to use pesticides in a way that secures not only residues below the MRLs but leaves the lowest possible amount of residues in the product at harvest. Danish control strategies against gray mold (*Botrytis cinerea*) and powdery mildew (*Sphaerotheca alchemillae*) in field-grown strawberries include one or more of the four fungicides fenhexamid, pyrimethanil, tolylfluanid, and kresoxim-methyl. Tolylfluanid has been used against *Botrytis* during the past two decades, whereas fenhexamid, pyrimethanil, and kresoxim-methyl are newer compounds, which have proven to be highly effective (*5*). To effectively control *Botrytis*, fungicide applications should primarily be made in the early developmental stages of strawberry flowers and fruits (*6*). In Denmark, fenhexamid, pyrimethanil, and tolylfluanid are registered for use in the strawberry flowering stage with preharvest intervals (PHI) of 10, 10, and 14 days, respectively, whereas kresoxim-methyl is registered for use during flowering but not later than June 1. In a field experiment conducted over 2 years, fungicide residues resulting from different use patterns were investigated. The objective of this work was to investigate the influence of the dose, number, and time of fungicide applications on residues in the strawberries harvested. This paper also describes the analytical method developed and validated for the detection of fenhexamid, pyrimethanil, tolylfluanid, DMST [*N*,*N*-dimethyl-*N*′-(4-methylphenyl)sulfamide, degradation product from tolylfluanid], and kresoxim-methyl using the liquid chromatography−mass spectrometry (LC-MS) technique. The harvested strawberries moreover served as material for studies, where the influence of processing factors such as rinsing

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Table 1. Fungicides Used in the Field Trials with Notification of the Amount of Active Ingredient (a.i.) in the Formulated Product, the Recommended Doses, and PHIs in Accordance with the Danish GAP

fungicide	formulated product (trade name)	company	fungicide content (a.i.) in formulated product	recommended rate of application (kg a.i. ha^{-1})	preharvest interval, PHI (days)
fenhexamid	Teldor WG 50	Bayer CropScience[a]	500 g kg^{-1}	0.75	10
pyrimethanil	Scala	BASF[b]	400 g L^{-1}	0.8	10
tolylfluanid	Euparen Multi	Bayer CropScience[a]	500 g kg^{-1}	1.5	14
kresoxim-methyl	Candit	BASF[b]	500 g kg^{-1}	0.1	c

[a] Monheim, Germany. [b] Ludwigshafen, Germany. [c] Application of kresoxim-methyl is allowed until the end of strawberry flowering but no later than June 1 according to Danish law.

Table 2. Fungicide Application Data from the Field Trials in 2001 and 2002

control strategy[a]	dose[b]	application rate (kg a.i. ha^{-1}) at time of treatments[c] (BBCH growth stage) 59	62	65	68	74	79	days between final application and 1st harvest for fungicide T	P	F
TTTT	*N*	1.500	1.500	1.500	1.500			21		
PPPP	*N*	0.800	0.800	0.800	0.800				21	
FFFF	*N*	0.750	0.750	0.750	0.750					21
TFTF	1/2*N*	0.750	0.375	0.750	0.375			28		21
	1*N*	1.500	0.750	1.500	0.750			28		21
TPTP	1/2*N*	0.750	0.400	0.750	0.400			28	21	
	1*N*	1.500	0.800	1.500	0.800			28	21	
PFPF	1/2*N*	0.400	0.375	0.400	0.375				28	21
	1*N*	0.800	0.750	0.800	0.750				28	21
TFTFPP	1/2*N*	0.750	0.375	0.750	0.375	0.400	0.400	28	10	21
	1*N*	1.500	0.750	1.500	0.750	0.800	0.800	28	10	21
TPTPFF	1/2*N*	0.750	0.400	0.750	0.400	0.375	0.375	28	21	10
	1*N*	1.500	0.800	1.500	0.800	0.750	0.750	28	21	10

[a] Letters T, P, and F indicate the fungicides tolylfluanid, pyrimethanil, and fenhexamid, respectively, used in the referred strategy. Strategies TTTT, PPPP, and FFFF were tested in 2001 only. [b] In three control strategies (TFTF, TPTP, and PFPF), a half dose (1/2*N*) was applied in 2002 only. [c] Corresponding to dates between May 21 and June 18 in 2001 and dates between May 15 and June 9 in 2002. In growth stage 59, the first flower petals are visible, whereas in growth stage 79 nearly all fruits have reached final size. For further description of the BBCH growth stages, see ref *10*.

and cooking on residue levels was investigated. Results from this work have been published by Christensen et al. (*7*).

Further literature reporting results from studies concerning residues of these fungicides in strawberries are limited. Angioni et al. (*8*) conducted comparable residue studies with pyrimethanil and fenhexamid, whereas Stensvand and Christiansen (*9*) reported data for tolylfluanid residues in greenhouse-grown strawberries.

MATERIALS AND METHODS

Field Experiments. Studies were conducted at the Danish Institute of Agricultural Sciences (DIAS), Research Centre Flakkebjerg (55°57′N 11°23′E). Plants of the strawberry cultivar Honeoye were planted in May 2000 in 5 m rows with 0.3 m between the plants and 1 m between the rows. A split plot scheme was used with four blocks (replications) divided in block halfs (whole plot units), each measuring 100 m^2 and consisting of 10 plots of two rows, i.e., 160 rows (plots), in the complete experiment. Fungicide treatments were initiated in the spring of 2001. **Table 1** lists information on the formulations used in the field trials.

The fungicides fenhexamid, pyrimethanil, and tolylfluanid were applied in each individual plot (row) with a hand-held boom sprayer, model Koldkaergaard from the Danish Agricultural Advisory Service (Aarhus, Denmark) and modified at the Research Centre Flakkebjerg (Slagelse, Denmark). A computer (PX Combi 821, Lykketronic A/S, Løgstør, Denmark) with a flow meter and a wheel-mounted rate indicator supported the sprayer to ensure a stable application. The boom sprayer was equipped with three nozzles (Hardi ISO F-03-110) equally distributed in an arch-formed boom covering one row and delivering 600 L ha^{-1} of spray mixture at 300 kPa. Applications of fenhexamid (F), pyrimethanil (P), and tolylfluanid (T) were conducted according to a range of different control strategies against gray mold disease with varying doses, numbers of applications, and times of application (**Table 2**). As an example, the control strategy named TFTFPP means that a total of six applications were made. The first and third were tolylfluanid applications, the second and fourth were fenhexamid applications, and the fifth and sixth were pyrimethanil applications. Moreover, in 2001, the fungicide kresoxim-methyl, used to control powdery mildew, was applied twice in normal dose to half of the whole plot units in the early stage of strawberry flowering (May 18 and 31) with a tractor-mounted boom sprayer from Hardi International (Taastrup, Denmark) mounted with ISO-F-03-110 flat fan nozzles.

Control strategies were partly changed in 2002 as compared to 2001 meaning that all strategies were conducted with two doses in 2002, the normal dose (N) as recommended in Denmark for strawberries and half of this (1/2N). Recommended rates of application appear in **Table 1**. For three strategies (TFTF, TPTP, and PFPF), the half-dose was used in 2002 only. Strategies with four applications with the same fungicide (TTTT, PPPP, and FFFF) were tested in 2001 only. In 2002, fungicide applications in half of the whole plot units were conducted with a windbreaking shield mounted on the hand-held boom sprayer. Kresoxim-methyl was not applied to any of the plots in 2002.

The ripened strawberries were harvested from the field on three dates, June 28 and July 3 and 6 in 2001 and June 19, 24, and 27 in 2002. The berries were picked from the mid 4 m of each row, and the sepals were removed. From each row, the total yield and incidence of berries infected with gray mold were recorded. Samples with a content of approximately 1000 g were subsequently stored frozen (−18 °C) in aluminum trays until homogenization, extraction, and analysis. Experienced personnel following approved protocols carried out all cultural, spraying, and harvesting practices.

Climatic Observations. Automatic loggers placed in plant height (10 cm above the ground) in the field continuously recorded the temperature (minimum and maximum values), while rainfall was

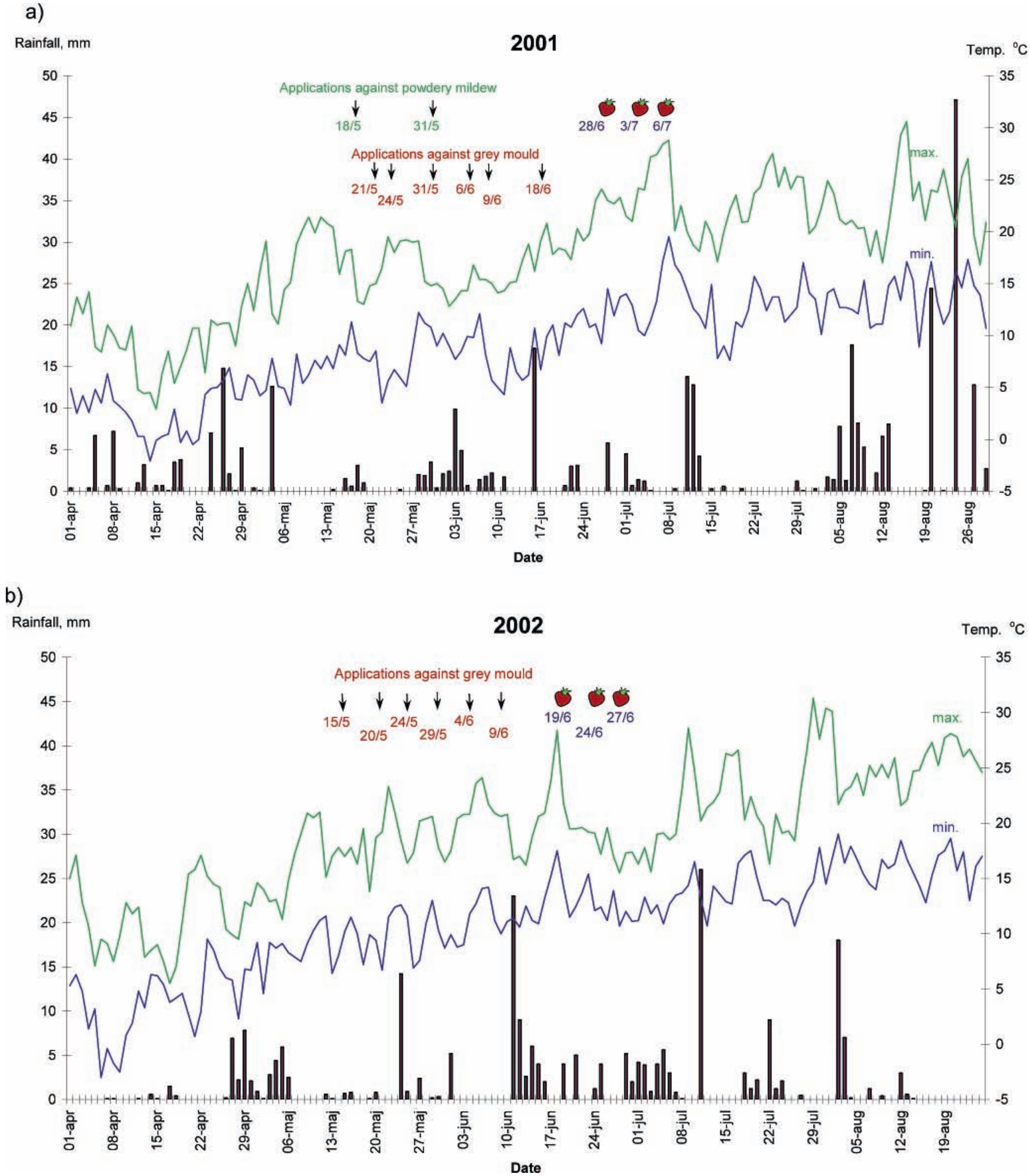

Figure 1. Temperature (minimum and maximum) and rainfall (mm) measured on a daily basis in the field during the growth seasons (**a**) 2001 and (**b**) 2002. Dates of fungicide applications and strawberry harvest are shown.

recorded on a daily basis at the local meteorological station of Research Centre Flakkebjerg. Records of temperature and precipitation are summarized in **Figure 1**.

Residue Sample Preparation. After thawing, each strawberry sample was homogenized in a glass beaker with an Ultra-Turrax homogenizer (IKA-Werke, Staufen, Germany) at 11000−24000 rpm for a few minutes. Ten grams of the homogenized sample was transferred to a Falcon 50 mL polypropylene conical tube (Becton Dickinson Labware, NJ) and 15 mL of methanol was added. The strawberry pulp was suspended in the methanol on a vortex mixer (IKA-Werke) and given 15 min in an ultrasonic bath (ABC-Sonic, ABC Hansen Engineering, Hørsholm, Denmark). Further extraction was performed on a Mastermixer (Heto-Holten, Allerød, Denmark) for 1.5 h. The sample was centrifuged for 15 min at 4000 rpm on a Sorwall RT7 (Newtown, CT) centrifuge, and the supernatant was transferred to another Falcon tube. Five milliliters of methanol was added to the first tube with the solid material, and the extraction was repeated. The total extracts were mixed and were ready for analysis.

Analytical Method. The determination of fungicide residues was made by LC-MS using a Hewlet Packard MSD 1100 (Palo Alto, CA). The chromatography was performed with gradient elution at a flow rate of 0.2 mL min^{-1} at 30 °C. The LC column was a 250 mm × 2.1

mm i.d. 5 μm, Hypersil BDS C18 (ThermoHypersil, PA). All reagents and solvents used were of gradient grade (Merck, Darmstadt, Germany), and high-purity water for the analysis was obtained in-house with a MilliQ system (Millipore Corporation, Bedford, MA). All pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and dissolved in 100% acetonitrile and diluted in 50% methanol:water. Gradient composition: initial conditions 40% A eluent (10 mM ammoniumacetate in water:methanol, 99:1), 60% B eluent (10 mM ammoniumacetate in water:acetonitrile, 10:90), linear gradient to 80% B in 12 min, 80% B kept for 1 min, and back to initial conditions in 1 min. The system was restabilized at 60% B for 8 min, and the total run time was 22 min. Detection was performed after electrospray ionization (ESI) with MSD 1100 in positive selected ion monitoring mode (SIM). The capillary voltage was 4000 V, the drying gas temperature was 350 °C, the drying gas flow was 10 L min^{-1}, and the nebulizer pressure was 40 psig.

Pyrimethanil was detected at *m*/*z* 200, fenhexamid was detected at *m*/*z* 302 and 304, and kresoxim-methyl was detected at *m*/*z* 267 and 238. Tolylfluanid was detected at *m*/*z* 238 and 240, and its degradation product DMST was detected at *m*/*z* 106 and 215. All had dwell times of 146 ms and fragmentor voltages of 60 V. Method validation included determination of linearity, estimation of detection limits, recovery, and day to day variation. The storage stability of the five pesticide compounds in the frozen strawberry homogenate was tested. Strawberry homogenate was spiked to a 50 μg kg^{-1} concentration level and stored at −18 °C. The stability samples were analyzed together with the real samples during up to 200 days of storage. For routine analysis, each batch consisted of 10 samples, one sample in duplicate, one blank sample, and one recovery sample at the 50 μg kg^{-1} level. Blank samples were extracts of strawberries grown without the use of pesticides.

Calculation of Half-Life. The half-life of pyrimethanil and fenhexamid was estimated by assuming that the recorded residues were a sum of the residues after each of the two applications of the fungicide and that both were assumed to follow first-order kinetics, using the following model, which is an extension and reformulation of the well-known formula for single applications (see, e.g., *11*):

$$Y = a \exp\left[\frac{\ln(0.5)}{h}t_1\right] + a \exp\left[\frac{\ln(0.5)}{h}t_2\right]$$

where Y is the residue, a is the amount of the fungicide at the time of application (from the actual application), h is the time until the initial amount is reduced to 50%, and t_1 and t_2 are the time since applications. The parameters a and h were estimated using the method of least squares.

Statistical Analysis. In 2001, the whole units were allocated to treatments with and without kresoxim-methyl to control mildew, whereas in 2002, the whole units were allocated to the use or not use of a shield mounted on the sprayer to protect against wind. In both years, the 20 subunits were allocated to 18 different combinations of dose and treatment strategies and two controls (i.e., without any spraying); in 2001, the 18 different treatments consisted of nine treatments each in two doses. To take into account both the split plot structure of the design and the correlations between the three repeated samplings of strawberries from the same plot, a linear mixed model (*12*) was used for analyzing the data. The estimates of the fixed effects, the variance components, and the correlations between observations from the same plot were calculated using the method of residual maximum likelihood (REML) (*12*). On the basis of this model, an analysis of variance table was set up to test for the effect of the treatment factors and interactions between these using F tests and to do pairwise comparisons using LSD values at the 5% level of significance. Because the standard deviations of the observed residues were approximately proportional to the mean, the observed residues were log-transformed before the statistical analysis in order to ensure variance homogeneity. As the effects of the factors may be expected to be proportional rather than additive, the use of log-transformed values seems reasonable.

As the chemical analyses are rather expensive, the data from the first year were used to examine how much the LSD values could be expected to increase if the strawberries from the same treatment in replicates 1 and 2 and from the same treatment in replicates 3 and 4 were bulked. They were bulked so that the chemical analyses were carried out on a subsample from the mixed bulk sample and thus saving half the number of chemical analyses. This was done by setting up equations describing the variance of the difference between treatments—taking into account the estimated variance components for blocks, whole plot units, and subplot units together with an assumed low (but realistic) variance component for the chemical analyses. This showed that for the comparisons of interest the LSD values would only increase marginally; therefore, such a procedure was applied in 2002. To see how the residues decreased from the first day of harvest to the second and third day, reduction factors were calculated as: $e^{\alpha_2-\alpha_1}$ and $e^{\alpha_3-\alpha_1}$ where α_1, α_2, and α_3 are estimates for harvest days 1, 2, and 3, respectively.

The results from the linear mixed model show that the residues vary from plot to plot. Therefore, to ensure that the consumers will not consume strawberries that exceed the MRL value, it is not sufficient to look at the estimated means. To calculate a risk that a sample from a single plot exceeded the MRL, we assumed that the log-transformed residues were normally distributed with the mean equal to the estimate for normal dose in the treatment with the highest mean and a variance given by the estimated variance components.

RESULTS AND DISCUSSION

Chemical Analysis. Ionization and fragmentation patterns were investigated. First, it was demonstrated that electrospray positive mode gave the highest signals. Next, the fragmentation pattern of the five compounds was determined at fragmentation voltages from 20 to 120 V. **Table 3** gives the most abundant fragments of the five compounds at 30, 60, and 90 V. Pyrimethanil gave only one main ion, the protonated molecular ion, at the three different voltages. Fenhexamid was more fragile and gave many fragments at all three voltages, but at 60 V, the protonated molecular ions of the two chloro isotopes, *m*/*z* 302 and 304, and their sodium adducts, *m*/*z* 324 and 326, were dominant. Kresoxim-methyl also gave many fragments. The molecular sodium adduct was the dominant ion at all three voltages, but at 60 V, the 267 fragment gave an abundant signal and was used for quantification. *m*/*z* 238, which was used for quantification of tolylfluanid content, was used as a qualifier ion for kresoxim-methyl. Tolylfluanid fragments very easily, and at 60 V fragmentor voltage, the protonated molecular ion only appears as 22% of the most abundant ion, *m*/*z* 238, which is the molecule minus one of the side chains. In the environment, tolylfluanid is degraded by first loosening the other side chain, the halogenated part, giving DMST. DMST gives at 60 V the protonated molecule, *m*/*z* 215 and *m*/*z* 106, which could be tolylfluanid without any of the two side chains. With 60 V fragmentor voltage, it was possible for four of the five compounds to include a quantifying ion and a qualifier ion, while pyrimethanil gave only one ion.

Homogenization, extraction, and analysis of the crude extract without any further purification steps gave satisfying detection limits and no interference in the chromatograms. Matrix suppression, which is often seen with electrospray ionization (*13*), was very limited and was expressed in acceptable recovery rates. Lower detection limits could be achieved by preconcentration of the extract if necessary. The five compounds were nicely separated, and the strawberry matrix gave no interferences. **Figure 2** displays a chromatogram of a strawberry sample at the 50 μg kg^{-1} spike level. The method validation data are noted in **Table 4**. Recoveries were adequate for all compounds. Recovery was about 70% for tolylfluanid, and the standard deviation was higher for tolylfluanid than for the other compounds. This was caused by the higher degradation rate for tolylfluanid, which gave slightly different recoveries depending on the time span from homogenization to analysis, which could

Table 3. Most Abundant Ions at Three Fragmentation Voltages

	30 V *m*/*z* (% relative abundance)	60 V *m*/*z* (% relative abundance)	90 V *m*/*z* (% relative abundance)
pyrimethanil	152640[a]	240640[a]	290560[a]
	200 (100), 201 (13)	200 (100), 201 (14)	200 (100), 201 (14)
fenhexamid	9222[a]	6325[a]	6815[a]
	105 (100), 187 (40), 302 (48), 304 (30), 324 (32), 326 (20)	105 (91), 241 (35), 269 (28), 302 (100), 304 (65), 324 (73), 326 (48)	105 (43), 241 (16), 302 (100), 304 (85), 324 (56), 326 (39)
kresoxim-methyl	45216[a]	69080[a]	71512[a]
	203 (22), 314 (90), 315 (20), 336 (100), 337 (20)	116 (25), 238 (24), 267 (61), 282 (45), 314 (26), 336 (100)	116 (23), 222 (33), 235 (34), 238 (30), 267 (31), 282 (39), 336 (100)
tolylfluanid	23016[a]	49448[a]	87472[a]
	215 (81), 238 (100), 240 (66), 347 (65), 349 (49), 369 (58), 371 (44)	137 (30), 215 (22), 238 (100), 240 (68), 347 (22), 349 (14), 369 (37), 371 (30)	106 (20), 137 (100), 238 (35), 240 (23), 369 (12)
DMST	90408[a]	50776[a]	89888[a]
	215 (100), 216 (12), 237 (49)	106 (100), 151 (43), 215 (99), 237 (79), 259 (26)	106 (100), 107 (18), 151 (11), 237 (16), 259 (16),

[a] Ion count of most abundant ion.

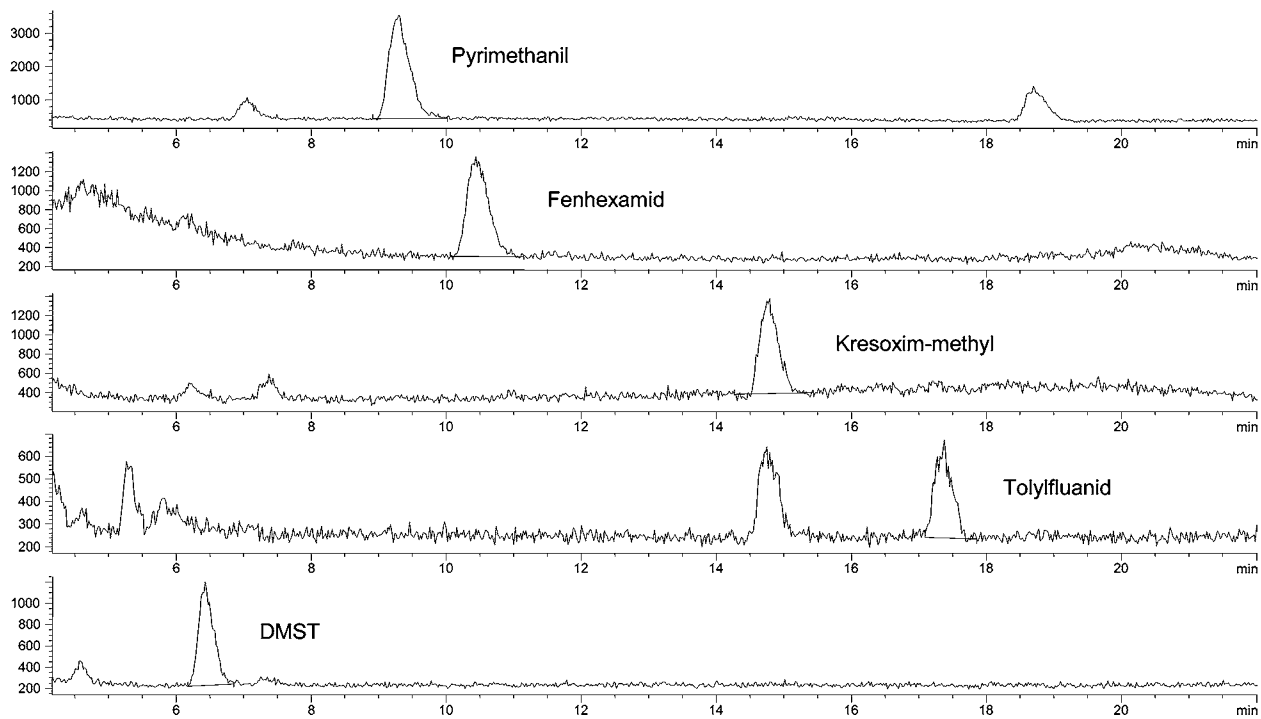

Figure 2. Chromatogram of blank strawberry sample fortified to 50 μg kg^{-1} of each pesticide: pyrimethanil, *m*/*z* 200; fenhexamid, *m*/*z* 302; kresoxim-methyl, *m*/*z* 267; tolylfluanid, *m*/*z* 238; and DMST, *m*/*z* 215.

fluctuate from day to day. Christensen et al. (*7*) and Fussel et al. (*14*) have investigated the stability of pesticides during processing, and these studies gave lower recoveries and inferior stability for tolylfluanid as seen in the present study while most pesticides were stable during processing. The calibration curves showed good linearity for all compounds with correlation coefficients above 0.996 in a concentration range between 10 and 1000 μg L^{-1}. The storage stability testing of the strawberry homogenates deviated from the real samples, which were frozen as whole berries. It was concluded that no significant loss could be determined after up to 180 days in the freezer for three of the pesticides while the fourth, tolylfluanid, was very degradable in the homogenate and gave fluctuating recoveries from 0 to 32% (**Figure 3**). Storage of whole berries is expected to give slower degradation rates with respect to lower enzymatic activity than in berries where the enzymes are released from the cells.

Effect of Application Strategies on Disease Incidence and Strawberry Yields. In both years, there were practically no gray mold attacks in the field; thus, no differences in disease incidence between sprayed and unsprayed plots nor between plots sprayed according to different control strategies were observed. As concerns the strawberry yields, the only significant effect was from the use of kresoxim-methyl in 2001, which resulted in a 10% yield increase as compared with plots not sprayed with the mildew fungicide.

Effect of Application Strategies on Fungicide Residues. The statistical analyses showed that the treatment with kresoxim-methyl against mildew (2001) and the use of a boom-mounted

Table 4. Residues in mg kg^{-1} Resulting from Applications of Pyrimethanil (P), Fenhexamid (F), and Tolylfluanid (T)[a]

		2001			2002			
control strategy	dose	P[b]	F[b]	T[b]	P[b]	F[b]	T[b]	DMST[b]
FFFF	*N*		0.135 b					
PFPF	*N*	0.147 cd	0.078 c		0.060 d	0.030 a		
PFPF	1/2*N*					0.008 b		
PPPP	*N*	0.493 ab						
TFTF	*N*		0.077 c	0.105 b		0.027 a	0.125 ab	0.030 b
TFTF	1/2*N*					0.012 b	0.024 d	0.011 c
TFTFPP	1/2*N*	0.348 b	0.035 d	0.038 cd		0.012 b	0.033 c	0.012 c
TFTFPP	*N*	0.660 a	0.072 c	0.102 b	0.244 b	0.032 a	0.130 b	0.036 ab
TPTP	*N*	0.260 bc		0.076 bc	0.163 c		0.104 a	0.039 a
TPTP	1/2*N*				0.078 d		0.035 c	0.012 c
TPTPFF	*N*	0.290 b	0.632 a	0.115 b	0.155 c	0.026 a	0.088 a	0.040 a
TPTPFF	1/2*N*	0.126 d	0.138 b	0.019 d	0.074 d	0.006 b	0.036 c	0.013 c
TTTT	*N*			0.481 a				
untreated		0.021 e	ND	ND	0.006e	ND	ND	ND
MRL[c]		1	1	5	1	1	5	
recovery (%)		89	95	88	92	86	68	84
LOD[d] (mg kg^{-1})		0.001	0.005	0.008	0.002	0.001	0.002	0.003
RSD_r (%)					2	1	3	4
RSD_R (%)		7	12	20	5	4	18	6

[a] Analyses of samples from the first harvest in 2001 and 2002, respectively. [b] Mean of four replicates. Values within a column followed by the same letter are not significantly different ($P \leq 0.05$; LSD test). [c] Danish MRLs during the study period. [d] See text.

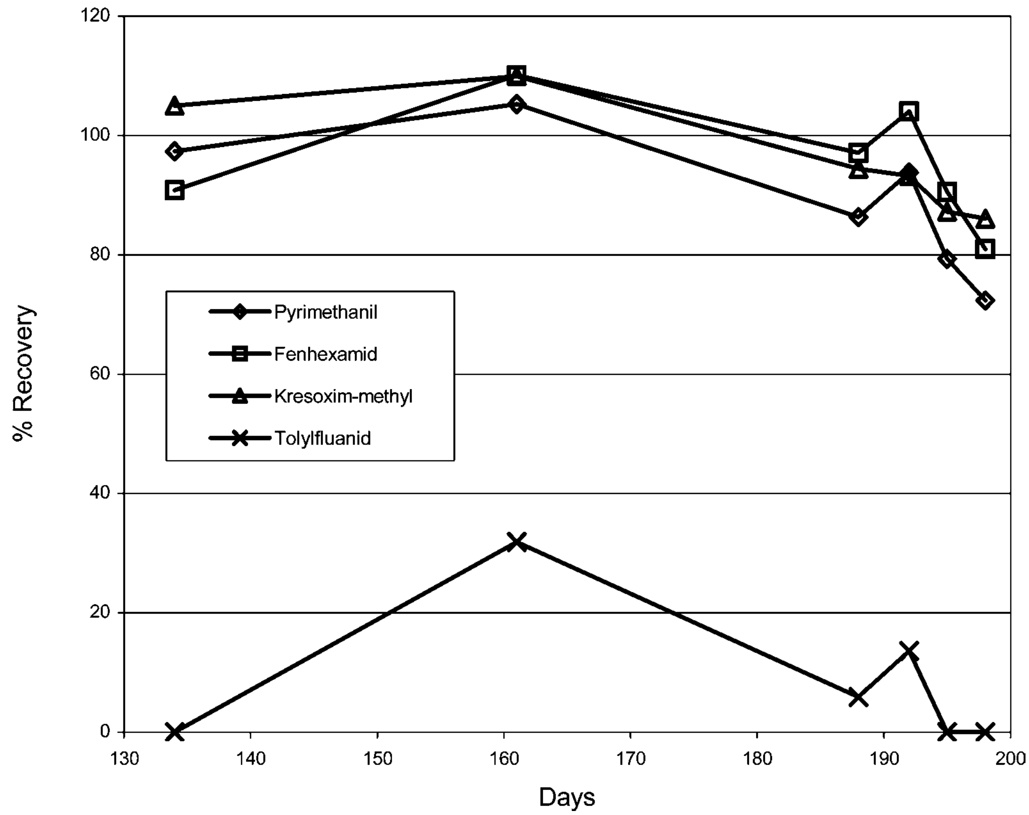

Figure 3. Stability of the four fungicides in homogenized strawberries at –18 °C. Fortification level at 50 μg kg^{-1}.

shield (2002) did not show effects on the fungicide residues shown here except in the following few cases: There was a significant three-way interaction, shield × dose × treatment strategy, for pyrimethanil, a significant, but very small three-way interaction, shield × dose × time, for fenhexamid, and a main effect of shield for DMST. Therefore, the effect of treatment against mildew and use of shield will not be commented on further and those factors just act as factorial replicates in the following presentations of results.

The average values of the fungicide residue data resulting from different disease control strategies, obtained by the analysis of strawberry samples from the first harvest date, are summarized in **Table 4**. For each of the fungicides, control strategies are listed in order of which contained the highest residue of the particular fungicide in 2001. It is indicated which residues within each year are significantly different on the basis of $LSD_{0.95}$ tests. Moreover, recovery, limit of detection (LOD), repeatability (RSD_r), and reproducibility (RSD_R) for each fungicide are noted in the three tables. LOD was calculated as three times the standard deviation on figures from analysis of 14 strawberry samples spiked with the analyzed pesticides. Repeatability is calculated as the within day variation while reproducibility is

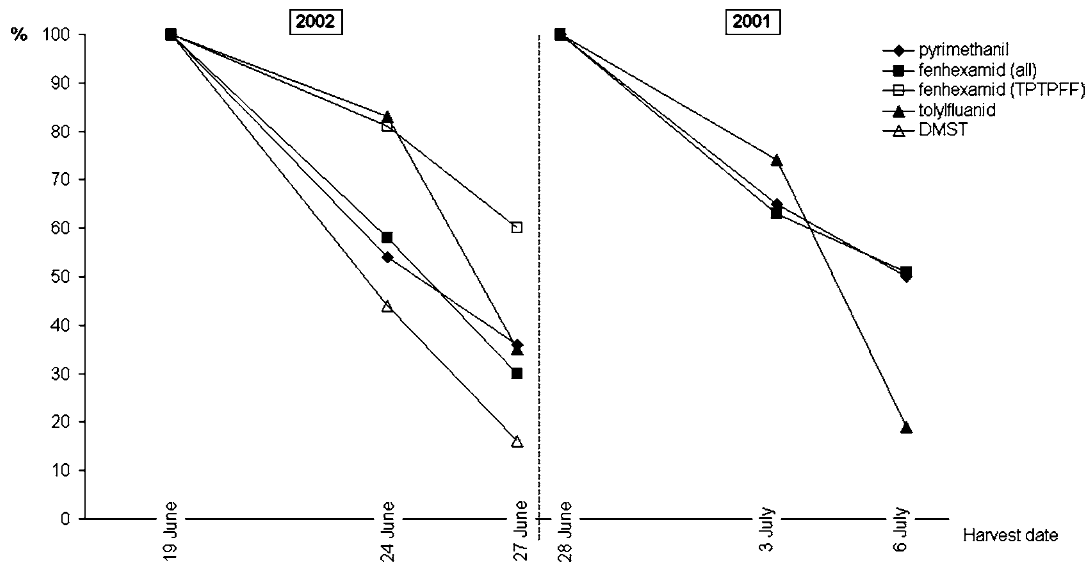

Figure 4. Relative reduction (%) of fungicide residues analyzed in strawberries from three harvest dates in 2001 and 2002. The residue levels at the first harvest date are set to 100% for each of the compounds.

calculated as the between days variation. In all strawberry samples analyzed, residues of pyrimethanil, fenhexamid, and tolylfluanid were below the MRL for all control strategies tested. In 2001, recommended rates gave residues of 0.66, 0.63, and 0.48 mg kg^{-1}, respectively, for pyrimethanil (10 days after treatment, = PHI), fenhexamid (10 days after treatment, = PHI), and tolylfluanid (21 days after treatment, = PHI plus 7 days). Residues resulting from the use of half rates of pyrimethanil and fenhexamid 10 days before harvest were 0.35 and 0.14 mg kg^{-1}, respectively. No residues of kresoxim-methyl were found in the samples from 2001, indicating that kresoxim-methyl residues had declined to a level well below the detection limit value within the 28 day period between the last application and the harvest. In 2002, the fungicide residues found in the samples were generally lower than in 2001. In this context, it is worth remarking that the monthly mean temperature in June 2002 was one degree higher than in June 2001 and the rain events in the 10 day period between the last fungicide application and the first day of harvest were markedly different in the two years of the field experiment. In 2001 (**Figure 1a**), it rained 13 mm between June 18 and 28. The following year, four times as much, 51 mm, was measured between June 9 and 19, of which 23 mm came the day after the last spraying (**Figure 1b**). In 2002, the highest concentrations of pyrimethanil and fenhexamid resulting from the use of recommended dose rates were 0.39 and 0.03 mg kg^{-1}, respectively, 10 days after the last application (PHI). Residues resulting from the use of half rates were 0.24 and 0.01 mg kg^{-1} for pyrimethanil and fenhexamid, respectively. For tolylfluanid and DMST, the highest residues 28 days after the last application (PHI + 14 days) were 0.13 and 0.04 mg kg^{-1} following the recommended dose rate, whereas half rate resulted in tolylfluanid and DMST residues of 0.03 and 0.01, respectively, in 2002.

The results indicate that even with the Danish PHIs, i.e., 10 days for pyrimethanil and fenhexamid and 14 days for tolylfluanid, which are relatively strict as compared with the PHIs in several other European countries, detectable residues cannot be avoided. The MRL values, 1 mg kg^{-1} for pyrimethanil and fenhexamid and 5 mg kg^{-1} for tolylfluanid, were not exceeded in any of the control strategies tested, but the intention of many Danish growers to be able to produce strawberries with no detectable amounts of pesticide residues is not a realistic issue. However, it is reasonable to advise growers to choose control strategies, which may lead to the lowest possible residues in the products at harvest time, as long as the strategies also secure a sufficient disease control.

Residue Levels in Relation to Harvest Date. The harvest period was of the same duration in both years, with the first strawberry picking conducted 10 days after the last fungicide application, the second picking 5 days later, and the third picking another 3 days later. Because of an earlier ripening in 2002, the harvest period was 10 days earlier than in 2001. **Figure 4** shows the percentage decline in residue levels at the three dates of harvest in 2001 and 2002, where the amount analyzed in berries from the first harvest date is set to 100%. During 5 days to the second harvest day and another 3 days to the third harvest day, pyrimethanil residues in comparison with the levels at the first harvest day were reduced to 65 and 50% in 2001 and 54 and 36% in 2002. Fenhexamid residues were reduced to 63% on the second and 51% on the third harvest day in 2001. In 2002, fenhexamid residues resulting from one particular control strategy (TPTPFF) declined significantly slower (to 81 and 60%) than was the case for the other strategies with fenhexamid, which were reduced to 58 and 30% on the second and third harvest days, respectively. Tolylfluanid reduction was slower the first 5 days and more rapid the following 3 days, reaching residue levels of 74 and 19% in 2001 and 83 and 35% in 2002.

The degradation of pyrimethanil and fenhexamid was assumed to follow first-order kinetics. On the basis of the results from 2001, where residues were relatively higher than in 2002, it was possible to calculate half-life values for pyrimethanil and fenhexamid (**Table 5**). Results from the use of control strategies with two applications of either of the fungicides were used. For pyrimethanil, it was the strategy TFTFPP with residues analyzed 10, 15, and 18 days after last application, the strategies TPTP and TPTPFF with residues analyzed 21, 26, and 29 days after last application, and the strategy PFPF with residues analyzed 28, 33, and 36 days after last application. For fenhexamid, it was the strategy TPTPFF with residues analyzed 10, 15, and 18 days after last application and the strategies TFTF and TFTFPP with residues analyzed 21, 26, and 29 days after last application. The calculated half-lives were 8.6 days for pyrimethanil and 5.0 days for fenhexamid. Results reported by Angioni et al. (*8*) showed half-lives of 4.8 and 7.7 days for pyrimethanil and fenhexamid, respectively.

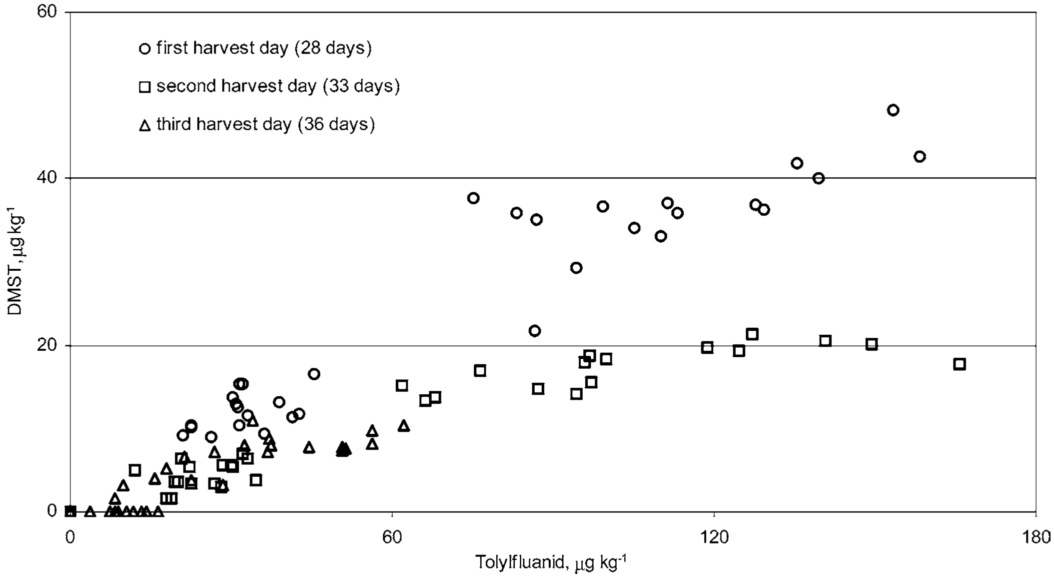

Figure 5. Dependence between tolylfluanid and DMST residues in strawberries harvested 28, 33, and 36 days after the last application of tolylfluanid.

Table 5. Residues Resulting from Two Applications with the Recommended Dose of Pyrimethanil or Fenhexamid in 2001 and the Calculated Half-Lives, $t_{1/2}$

time after first and second application (days)	residue (mg kg^{-1})	
	pyrimethanil	fenhexamid
19–10	0.660	0.632
24–15	0.411	0.295
27–18	0.317	0.281
34–21	0.275	0.076
39–26	0.147	0.049
38–28	0.147	
42–29	0.147	0.041
43–33	0.127	
46–36	0.060	
$t_{1/2}$ (days)	8.6 ± 0.46	5.0 ± 0.64

The metabolism of tolylfluanid in plants proceeds through degradation to DMST, which is the main metabolite. As DMST has a similar toxicity as compared to tolylfluanid, it is regarded as a residue of concern for risk assessment and monitoring purposes (*14*). **Figure 5** displays the correlation between DMST and tolylfluanid residues in the strawberries at the three harvest dates. The DMST:tolylfluanid ratio appears to be higher at the first day of harvest as compared to the second and third day of harvest, indicating a relatively slower degradation of tolylfluanid as compared with the degradation of DMST, which corresponds well with the reduction patterns shown in **Figure 4**. At the third day of harvest, the concentration level of tolylfluanid as well as DMST had decreased and the highest concentrations of tolylfluanid were about 60 μg kg^{-1} and DMST about 10 μg kg^{-1}.

Recommended Fungicide Strategies. As mentioned earlier, there were practically no fungal diseases found in the strawberries in the two years of the field experiment. As such, it is difficult to recommend one fungicide strategy for another, when the resulting disease control cannot be addressed, not least with the use of reduced doses. Keeping this in mind, the residue results obtained in this work confirm that the Danish MRL of 1 mg kg^{-1} for fenhexamid and pyrimethanil is reasonable when the PHI is 10 days. In other EU countries, e.g., Sweden, Germany, and the United Kingdom, fenhexamid and pyrimethanil can be applied, respectively, 7, 3, and 1 day before harvest, but then, the MRLs in these countries are also higher: 2, 3, and 5 mg kg^{-1} for fenhexamid and 5 mg kg^{-1} for pyrimethanil.

Statistic Probability of Having a MRL Exceedence. Although none of the strawberry samples analyzed in this experiment actually contained residues above the MRL, it is also a fact that the residue variability between individual samples is significant and inevitable, as described by Hill and Reynolds (*17*). The calculated risk of obtaining that the MRL was exceeded in strawberries from one of the plots at the first day of harvest was high in 2001 and low in 2002. For the two fungicides pyrimethanil and fenhexamid, the approximate estimated risks were between 14 and 27% in 2001 and less than 0.000035% in 2002. This reflection clearly demonstrates that risk assessment of pesticides can be a statistically difficult problem because residues occur only occasionally and the exposure levels are highly variable as discussed recently by Paolo et al. (*16*). Although modeling of pesticide fate is getting still more refined, it is likely that the move toward probabilistic risk assessment methods will require some research support to determine the level and potential for pesticide recovery and human exposure.

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