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Method Development and Fate Determination of Pesticide-Treated Hops and Their Subsequent Usage in the Production of Beer

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The fate of residues of seven agrochemicals (chlorfenapyr, quinoxyfen, tebuconazole, fenarimol, pyridaben, and *E*- and *Z*-dimethomorph) from the treatment on hops to the brewing of beer was studied. First, a multi-residue analytical method was developed for the determination of pesticide residues in spent hops, trub, wort, and beer. Each matrix was validated over at least two levels of fortification, for all seven compounds, in the ranges 0.05−5.0, 0.001−1.0, 0.001−0.05, and 0.0005−1.0 ppm for spent hops, trub, wort, and beer, respectively. Recoveries ranged from 73 to 136%. Second, the matrixes prepared from hops, which were treated under commercial practices with each compound, were analyzed using the method developed. The use of treated hops resulted in the carryover of 0.001 ppm of tebuconazole, 0.008 *Z*-dimethomorph, and 0.005 ppm of *E*-dimethomorph into the wort. The bulk of the remaining residues of all seven compounds was found on the spent hops. Following fermentation, all compounds were found in levels less than 0.0005 ppm in beer, except *Z*- (0.006 ppm) and *E*-dimethomorph (0.004 ppm). Third, when all seven pesticides were spiked prior to the pitching of yeast into clean wort, most of the nonpolar compounds (chlorfenapyr, quinoxyfen, and pyridaben) partitioned into the organic material (trub) which settled to the bottom, while the more polar compounds (fenarimol, tebuconazole, and *E*- and *Z*-dimethomorph) were generally distributed evenly between the beer and the trub.

KEYWORDS: Beer; gas chromatography/mass spectrometry; hops; pesticides

INTRODUCTION

Beer has been an important beverage for reportedly over 8,000 years (*1*). Over this span of time the recipe for beer has had many additions and substitutions, yet throughout these changes the basic formula has remained the same. Sometime between the sixth and ninth centuries, the first usage of hops (*Humulus lupulus*) in beer was reported (*1, 2*). Hops, which have been known to be a natural preservative, were used to increase the shelf life of beer, as well as to add flavor. Even though the need for hops as a preservative decreased withthe development of refrigeration and pasteurization, hops still play a major role in the beer brewing process as a source of bitterness, flavor, and aroma.

Currently, primary production of hops in the U.S. occurs in the Pacific Northwest region, in states such as Idaho, Oregon, and Washington (*3*). In 1999, 34,260 acres of hops were produced (*4*). Hops have several pests including hop aphid, two-spotted spider mite, and lepidoptera (*4, 5*). Until recently, downy mildew was the most serious disease for hops, but within the past few years, powdery mildew outbreaks have raised some concern with hop growers (*1, 5*). To prevent these pests, growers have requested pesticide registrations from the U.S. Environmental Protection Agency (USEPA) on compounds to be used on hops. As a result of these requests, the U.S. Department of Agriculture Interregional Research Project No. 4 (USDA IR-4) program conducted magnitude-of-residue field trials on hops for several compounds, including chlorfenapyr, quinoxyfen, tebuconazole, fenarimol, pyridaben, and dimethomorph (*6−11*).

Typically, the aforementioned compounds are applied several times during the growing season and have a short pre-harvest interval, which can lead to high residue levels on the raw agricultural commodity (*6−11*). The residue levels are intentionally high to provide continued crop protection, yet these elevated residues may carry over into the beer during the brewing process. Previous work has been conducted on the extraction of pesticides from hops by liquid CO_2 during hop extract production, but the fate of the pesticides during the brewing process was not covered (*12*). Papers have been published on the fate of organophosphates, carbamates, pyrethroids, triazines, and organochlorines found on raw materials (malt and hops) used in the brewing process (*13−15*). However, little work has been conducted on the fate of the compounds mentioned above in the brewing process itself. Therefore, it is of importance to understand the potential exposures of these pesticides when comparing the benefits of a higher quantity/quality of crop versus the risks of exposure.

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Table 1. Analytical Pesticides

compound	purity	log K_{ow}	supplier	location
chlorfenapyr	99.7%	4.83	Cyanamid	Princeton, NJ
quinoxyfen	99.8%	4.66	DowAgro	Midland, MI
tebuconazole	94.7%	3.70	Bayer	Kansas City, MO
fenarimol	99.4%	3.69	DowAgro	Indianapolis, IN
pyridaben	99.7%	6.37	BASF	Limbergurhof, Germany
dimethomorph	97.6%	2.73/2.63 (*Z*/*E*)	Cyanamid	Princeton, NJ
oxyfluorfen[a]	99.9%	N/A	Rohm & Haas	Philadelphia, PA

[a] Oxyfluorfen served as an internal standard.

Table 2. Sample Location and Application Rates

compound	rate (lbs/acre)	number of applications	location
chlorfenapyr	0.310	3	Parma, ID
quinoxyfen	0.134	4	Parma, ID
tebuconazole	0.225	4	Hubbard, OR
fenarimol	0.055	4	Hubbard, OR
pyridaben	0.500	2	Prosser, WA
dimethomorph	0.400	7	Parma, ID
untreated	N/A	N/A	Parma, ID

In the present study, a new method using gas chromatography−mass spectrometry for the detection of seven pesticides in spent hop, trub, wort, and beer samples was developed to determine the fate of the pesticides on hops during the brewing process.

EXPERIMENTAL PROCEDURES

Pesticides. Chlorfenapyr, dimethomorph, quinoxyfen, fenarimol, tebuconazole, pyridaben, and oxyfluorfen were all analytical grade standards stored at −20 °C. See **Table 1** for purity, origin, and K_{ow} values (octanol/water partition coefficients).

Materials. All solvents and reagents were pesticide grade.

Stock and Fortification Solution Preparation. A stock solution (1.0 mg/mL) was prepared for each analytical standard in acetone. A mixed 100 μg/mL fortification standard (all compounds except oxyfluorfen) was prepared by taking a 5-mL aliquot of each 1.0 mg/mL stock solution and diluting the mixed aliquots in a 50-mL volumetric flask with acetone. The 100 μg/mL mixed solution was then serially diluted to make a 10 and a 1 μg/mL solution. An internal standard spiking solution was prepared by taking a 500-μL aliquot of the 1.0 mg/mL oxyfluorfen stock solution and diluting the aliquot in a 50-mL volumetric flask with 0.1% corn oil in ethyl acetate, resulting in a 10 μg/mL solution. A 5-mL aliquot of the 10 μg/mL solution of oxyfluorfen was then diluted in a 50-mL volumetric flask with 0.1% corn oil in ethyl acetate, resulting in a 1 μg/mL solution. All stock, fortification, and internal standard solutions were stored at −20 °C in the dark until use.

GC Calibration Solution Preparation. GC calibration solutions were prepared by adding 250 μL of the 10 μg/mL mixed fortification solution to volumetric flasks of 25, 50, 100, and 200-mL capacities. Aliquots of 62.5, 125, 250, and 500 μL of the 10 μg/mL internal standard solution were added to the 25, 50, 100, and 200-mL volumetric flasks, respectively. The flasks were then diluted to the mark with 0.1% corn oil in ethyl acetate, resulting in 100, 50, 25, and 12.5 pg/μL solutions, respectively (25 pg/μL of internal standard in each). All calibration solutions were stored at 5 °C in the dark until use.

Sample Collection from Fields. A total of 7 hop samples (6 treated, one for each pesticide of interest, and 1 untreated control) were collected from IR-4 field trial sites in Oregon, Idaho, and Washington. See **Table 2** for pesticide and application rates. Following collection, the hop samples were dried in a manner consistent with commercial drying methods (heated air kilns) and transferred, in a frozen state, to our facility.

Laboratory-Scale Fermentation Study. Cold untreated wort (4 L) was transferred to an 8-L glass carboy and 4-mg of each pesticide was added, resulting in a 1 ppm solution. To the spiked wort, yeast slurry was added and vigorously mixed. Following mixing, a sample (100 mL aliquot) was taken to represent time zero ($T = 0$), and subsequent samples were taken over 35 days. Once the last sample had been removed, the remaining wort was drained and the trub was transferred to a 1000-mL Erlenmeyer flask for further analysis.

Laboratory-Scale Brewing Trials. Ground malt (24 kg) was mashed with 10.4 L of water in a 40-qt cooler at 68 °C for 78 min. The resulting mash was then sparged with 20.8 L of water at 81 °C over 30 min. The sweet wort was then transferred to a 6.5-gal stainless steel kettle and brought up to boiling for 30 min. After 30 min of boiling, 200 g of dried hops (untreated hops were used for method validation studies, and treated hops were used for the pesticide fate determination study) was added and the boiling was continued for another 60 min. Upon the cessation of boiling, the wort was chilled to 24 °C and the spent hops were removed and refrigerated until analysis could be attempted. A sample (100-mL aliquot) was taken from the wort for analysis prior to fermentation.

Chilled wort was transferred to a 6-gal glass carboy, and yeast slurry was pitched in with vigorous mixing. For the treated hops test, an aliquot of 100 mL was taken from the fermentation carboy just after the addition of yeast. This sample represents time zero ($T = 0$) for a 38-d monitoring of residues in the fermenting wort/young beer.

Sample Preparation for Beer and Wort. Beer and wort samples (100 mL) were transferred into 250-mL Erlenmeyer flasks. For spike and recovery studies, untreated beer and wort samples were fortified at this point with a known amount of each pesticide of interest. The samples were then diluted with 100 mL of water and mixed. If the sample had suspended solids, the diluted sample was filtered, under reduced vacuum, with a Büchner funnel fitted with Whatman 541 filter paper covered with a thin layer of Celite 545 (1 teaspoon, Fisher Scientific, Fair Lawn, NJ).

An Oasis HLB cartridge (0.5 g/6 mL, Waters Corporation, Milford, MA) was conditioned, under mild vacuum, with 2 column volumes (CV) of ethyl acetate followed by 2 CV of methanol. The cartridge was then prepared for the sample loading with 2 CV of water. A 75-mL reservoir was attached to the cartridge and the sample was loaded under mild vacuum (drip rate of 1−2 drops/second). Once the entire sample was loaded, the Erlenmeyer flask was rinsed with 3 × 5 mL of water and the rinses were loaded onto the cartridge. The cartridge was then washed with 2 CV of 40% methanol in water and allowed to briefly dry. The compounds of interest were eluted with 2 CV of 80% ethyl acetate in hexane and transferred to a 100-mL round-bottom flask for sample concentration via rotary evaporation, under mild vacuum (water bath at 40 °C). Following evaporation, the sample was redissolved into 5 mL of 40% ethyl acetate in hexane for further cleanup.

An Iso-lute aminopropyl solid phase extraction (SPE) cartridge (1 g/6 mL, International Sorbent Technology, Glamorgan, U.K.) was conditioned with 1 CV of 40% ethyl acetate in hexane. The conditioned SPE was placed into a 100-mL round-bottom flask and the redissolved sample was loaded (all eluant was collected). The original 100-mL round-bottom flask was rinsed with 5 mL of 40% ethyl acetate in hexane and was loaded on to the SPE. Once the 40% ethyl acetate in hexane washes had passed, a 20-mL reservoir was attached to the SPE, and the remaining compounds were eluted with 15 mL of 80% ethyl acetate in hexane. Solvents were allowed to pass through the SPE by gravity. After elution, the sample was concentrated to near dryness. An internal standard (oxyfluorfen) was added at this point, for injection reproducibility, and the sample was diluted accordingly with 0.1% corn oil in ethyl acetate to facilitate analysis by gas chromatography−mass selective detection (minimum sample volume was 2 mL).

Sample Preparation for Spent Hops and Trub. A modification (extraction, liquid partition, and GPC) of the method developed for the analysis of dimethomorph in dried hops was used for spent hops and trub (*10*). Spent hop samples (2 g, dry weight equivalent) were measured out into 1-qt stainless steel Waring blender cups (Waring Corporation, Winsted, CT), and trub samples (50 mL of trub slurry) were measured out into a 250-mL Erlenmeyer flask for extraction by an Ultra-Turrax T-25 (Janke & Kunkel, West Germany). For spike and

recovery studies, untreated spent hop and trub samples were fortified at this point with a known amount of each pesticide of interest. The sample was then blended with 100 mL of acetone for two minutes. The blended sample was filtered, under vacuum, using a Büchner funnel fitted with a Whatman 41 filter paper covered with a small layer of Celite 545 (1 teaspoon). The resulting filter cake was transferred back to the blending cup and blended for another 2 min with 100 mL of acetone and re-filtered. The blender cup was rinsed with 50 mL of acetone, and the rinsate was added to the filter cake.

After filtration, the entire sample was transferred to a 1000-mL separatory funnel that contained 500 mL of water and 50 mL of saturated sodium chloride solution. The filtration flask was rinsed with 100 mL of dichloromethane, and the rinsate was added to the separatory funnel, which was then shaken for 2 min. The phases were then allowed to separate, and the lower organic layer was drained through a funnel plugged with glass wool and sodium sulfate into a 500-mL round-bottom flask. The remaining aqueous layer was re-extracted with another 100 mL of dichloromethane for two more min and pooled with the first partition of dichloromethane. The sodium sulfate was rinsed with 25 mL of dichloromethane into the 500-mL round-bottom flask. The sample was then concentrated to dryness on a rotary evaporator under vacuum (water bath ca. 40 °C). After concentration, the sample was dissolved into 10 mL of 1/1 (v/v) dichloromethane/cyclohexane for cleanup via gel permeation chromatography (GPC).

The GPC system consisted of a Kontes Chromaflex gel permeation column (Kontes, Vineland, NJ), a Foxy 200 X-Y fraction collector (Isco, Inc., Lincoln, NE), and a Benchmate II Workstation (Zymark Corporation, Hopkinton, MA). The Benchmate was programmed to automatically weigh, vortex, and filter (PTFE, 0.45-μm Millipore filter disk, Millipore Corporation, Bedford, MA) each sample prior to injection (5-mL sample loop is equal to $^1/_2$ the original sample on column) onto the GPC column. The column was 62 cm × 2.5 cm i.d. packed with 200/400 mesh S-X3 resin (Bio-Rad, Richmond, CA) to a bed length of 50 cm. The GPC mobile phase consisted of dichloromethane/cyclohexane (1/1, v/v), with a flow rate of 5 mL/min. Once the sample was loaded onto the column, the fraction collector was programmed to discard the first 120 mL (24 min) of eluate and then collect the next 80 mL (16 min) of eluate into a 250-mL TurboVap tube (Zymark Corporation, Hopkinton, MA). The GPC column was regenerated with 250 mL (50 min) of mobile phase prior to the next sample injection.

After GPC cleanup, the samples were placed into a TurboVap II concentration workstation and were concentrated to dryness with dry nitrogen (water bath at 45 °C). The sample residues were then redissolved in 5 mL of 40% ethyl acetate in hexane.

Mega Bond-Elut aminopropyl SPE columns (5 g/20 mL, Varian, Harbor City, CA) were preconditioned with 1 CV of 40% ethyl acetate in hexane. When the solvent reached the top of the packing, the sample was loaded onto the SPE and the eluate was collected in a 100-mL round-bottom flask. Flasks used for sample concentration, prior to SPE cleanup, were rinsed with 5 mL of 40% ethyl acetate in hexane, and the rinsate was added to the SPE. Following the addition of the rinsate, the SPE was fitted with a 75-mL reservoir, and the remaining compounds were eluted with 10 mL of 40% ethyl acetate in hexane, followed by 25 mL of 80% ethyl acetate in hexane. Solvents were allowed to pass through the SPE by gravity. After elution, the sample was concentrated to near dryness. An internal standard (oxyfluorfen) was added at this point, for injection reproducibility, and the sample was diluted accordingly with 0.1% corn oil in ethyl acetate to facilitate analysis by gas chromatography−mass selective detection (minimum sample volume was 2 mL).

Sample Analysis. Sample analysis was conducted with a Hewlett-Packard (HP) 6890-5973 GC−MSD (Hewlett-Packard, Avondale, PA) equipped with a 15 m × 0.25 mm I. D. ($d_f = 0.25$ μm) DB-XLB column (J&W Scientific, Folsom, CA). The MSD source (held at 230 °C) was operated in positive electron ionization mode, while the mass filter quadrupole (held at 150 °C) was operated in selective ion monitoring (SIM) mode. See **Table 3** for selected ions and retention times for each pesticide. The injector and GC−MSD transfer line were operated at 250 and 280 °C, respectively. An HP 6890 series autoinjector was used to inject 1 μL of sample in pulsed splitless mode (50 psi for 1 min, injector purge at 0.95 min). The injection port was fitted with a Restek Siltek Cyclo double gooseneck inlet liner (Restek Corp., Bellefonte, PA). The oven temperature started at 190 °C and was then programmed at 20 °/min to 280 °C and held for 5 min. Throughout the run the carrier gas (helium) was maintained at 2.0 mL/min. Pesticide concentrations were calculated by comparing the ratio of peak area response of the analyte over the internal standard in samples to those of calibration standards.

Table 3. Selected Ion Monitoring (SIM) and Retention Times for Pesticides

compound	MS fragments (*m/z*)	R_t (min)
oxyfluorfen	252 (100%), 300 (27%), 361[a] (M^+ 33%), 363[a] (M^++2 16%)	2.95
chlorfenapyr	59 (100%), 247 (8%), 406[a] (M^+ 5%), 408[a] (M^++2 5%)	3.22
quinoxyfen	237 (100%), 272[a] (37%), 307[a] (M^+ 30%), 309[a] (M^++2 19%)	4.13
tebuconazole	83 (42%), 125 (96%), 250[a] (M^+ 100%), 252[a] (M^++2 35%)	4.52
fenarimol	139 (100%), 295[a] (18%), 330[a] (M^+ 45%), 332[a] (M^++2 21%)	6.12
pyridaben	147 (100%), 309[a] (9%), 364[a] (M^+ 7%), 366[a] (M^++2 4%)	6.70
Z-dimethomorph	165 (30%), 301[a] (100%), 387[a] (M^+ 31%), 389[a] (M^++2 11%)	8.94
E-dimethomorph	165 (30%), 301[a] (100%), 387[a] (M^+ 31%), 389[a] (M^++2 11%)	9.29

[a] Used for SIM.

Table 4. Beer Method Validation Results (% recovery)[a]

compound	1.0 ppm	0.1 ppm	0.01 ppm	0.001 ppm	0.0005 ppm
chlorfenapyr	84 ± 6	101 ± 3	95 ± 2	98 ± 4	95 ± 8
quinoxyfen	82 ± 7	96 ± 2	97 ± 3	97 ± 6	95 ± 6
tebuconazole	94 ± 3	107 ± 4	110 ± 3	129 ± 2	131 ± 5
fenarimol	94 ± 6	106 ± 2	104 ± 2	102 ± 3	91 ± 5
pyridaben	70 ± 4	98 ± 3	102 ± 2	109 ± 4	108 ± 6
Z-dimethomorph	93 ± 7	103 ± 4	99 ± 4	94 ± 3	87 ± 4
E-dimethomorph	94 ± 6	104 ± 1	110 ± 2	91 ± 4	92 ± 11

[a] Number of replicates at each level is 6.

Table 5. Wort Method Validation Results (% recovery)[a]

compound	0.5 ppm	0.01 ppm	0.001 ppm
chlorfenapyr	97 ± 4	95 ± 5	97 ± 2
quinoxyfen	97 ± 4	96 ± 4	98 ± 5
tebuconazole	103 ± 4	108 ± 3	105 ± 4
fenarimol	101 ± 5	102 ± 5	94 ± 3
pyridaben	80 ± 5	90 ± 2	90 ± 3
Z-dimethomorph	98 ± 5	96 ± 4	94 ± 4
E-dimethomorph	101 ± 7	98 ± 6	93 ± 6

[a] Number of replicates at each level is 4.

RESULTS AND DISSCUSSION

Recovery Efficiencies of Pesticides from Spent Hops, Trub, Wort, and Beer. The method developed in the present study was validated for the compounds of interest in spent hops, trub, wort, and beer. Each matrix was evaluated at a minimum of two levels of fortification with at least 4 replicates (**Tables 4**−**7**). Spent hop matrix spikes yielded recoveries ranging 73−116% with a limit of quantitation of 0.050 ppm and a limit of detection of 0.023 ppm. Trub matrix spikes recoveries ranged 77−107% with a limit of quantitation of 0.001 ppm and limit of detection of 0.0005 ppm. Recovery spikes using wort matrix ranged 74−113% with limits of quantitation and detection of 0.001 and 0.0005 ppm, respectively, whereas beer spike recoveries ranged 66−139% with limits of quantitation and detection of 0.0005 and 0.00023 ppm, respectively. Limit of quantitation (LOQ) was defined as the lowest fortification level attempted and the limit of detection (LOD) was defined as 10% below the smallest standard in the standard curve (12.5 pg/μL). Overall the recoveries from each matrix were acceptable and

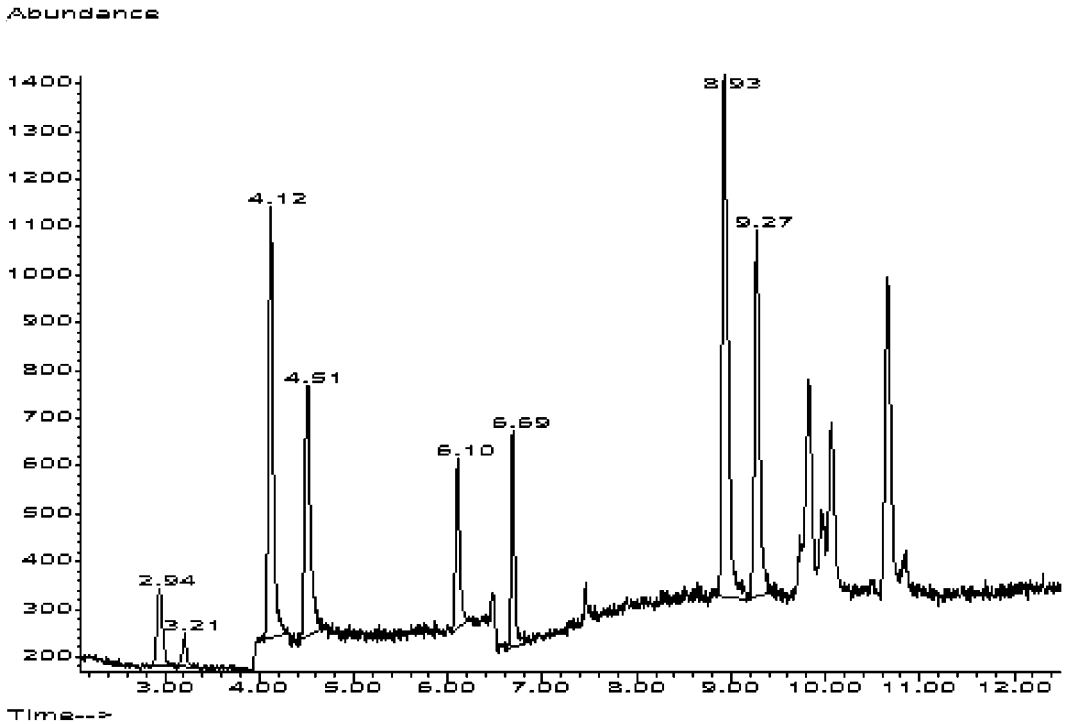

Figure 1. Selected ion chromatograms of 25 pg/μL of oxyfluorfen (m/z = 361, R_t = 2.94 min), internal standard in 12.5 pg/μL of chlorfenapyr (m/z = 408, R_t = 3.21 min), quinoxyfen (m/z = 309, R_t = 4.12 min), tebuconazole (m/z = 250, R_t = 4.51 min), fenarimol (m/z = 332, R_t = 6.10 min), pyridaben (m/z = 366, R_t = 6.69 min), *Z*-dimethomorph (m/z = 389, R_t = 8.93 min), and *E*-dimethomorph (m/z = 389, R_t = 9.27 min).

Table 6. Spent Hop Method Validation Results (% recovery)[a]

compound	5.0 ppm	0.5 ppm	0.05 ppm
chlorfenapyr	99 ± 2	103 ± 4	90 ± 5
quinoxyfen	97 ± 1	100 ± 2	99 ± 4
tebuconazole	96 ± 1	93 ± 4	85 ± 3
fenarimol	98 ± 2	94 ± 4	74 ± 1
pyridaben	96 ± 2	92 ± 4	93 ± 4
Z-dimethomorph	98 ± 2	97 ± 5	112 ± 4
E-dimethomorph	98 ± 3	100 ± 4	103 ± 7

[a] Number of replicates at each level is 4.

Table 7. Trub Method Validation Results (% recovery)[a]

compound	1.0 ppm	0.001 ppm
chlorfenapyr	92 ± 6	99 ± 3
quinoxyfen	98 ± 3	107 ± 1
tebuconazole	103 ± 4	102 ± 3
fenarimol	101 ± 2	91 ± 3
pyridaben	88 ± 7	99 ± 7
Z-dimethomorph	99 ± 3	88 ± 4
E-dimethomorph	100 ± 4	86 ± 7

[a] Number of replicates at each level is 4.

show that the method developed is appropriate for the simultaneous determination and confirmation of agrochemical residues in the beer brewing process. See **Figures 1−3** for representative chromatograms.

Pesticide Fate During the Fermentation Process. Pesticides could reportedly persist through the fermentation process in winemaking (*16*−*18*). Therefore, it was of importance to determine whether the compounds of interest in this study would survive beer fermentation. Samples were taken over a 35-day monitoring period to track the residue levels (**Figure 4**). Upon the spiking and pitching of the yeast, a dramatic drop in chlorfenapyr, quinoxyfen, and pyridaben residues were observed in the first time point (T = 0). Alternatively, tebuconazole, fenarimol, and both *Z* and *E*-dimethomorph had relatively high residue recoveries. Of the pesticides added, tebuconazole, fenarimol, and both *Z* and *E*-dimethomorph remained in the young beer after 35 days (55%, 41%, 70%, and 75% of total residues, respectively). The other three compounds were below the limit of quantitation (0.0005 ppm). Chlorfenapyr, quinoxyfen, and pryidaben were found in the trub at 34, 62, and 43% of total residues, respectively. In addition, residues of tebuconazole (58%), fenarimol (48%), *Z*-dimethomorph (22%), and *E*-dimethomorph (23%) were also found and accounted for remaining residues lost from the wort solution, such that the sum of the values from the last monitoring sample and the trub sample were approximately 100% of the total residues added.

These results would suggest that there is a relationship between water solubility (K_{ow}) and the amount of pesticide found in the aqueous wort/young beer and in the trub. The compounds with the lower K_{ow} values, such as *E*-dimethomorph (K_{ow} = 2.63) would be expected to be in relatively high concentration in the wort/young beer, when compared to pyridaben (K_{ow} = 6.37), which preferably partitioned into the lipophilic trub (*19*). A correlation equation was calculated by plotting log K_{ow} (x values) of each compound versus the corresponding percent recovery of residues in the young beer (y values). The resulting equation was $y = -23.3x + 130$ ($r^2 = 0.814$). This partitioning correlation was comparable to observations made for pesticide carryover from malt (*15*). Also, the lower recovery of chlorfenapy, quinoxyfen, and pyridaben in the trub would suggest a secondary route of loss. To address this, the carboy used in the fermentation trial was rinsed several times with ethyl acetate to determine if any of the more nonpolar compounds adsorbed to the glass wall, which has been suggested by Miyake as a possible route of loss (*15*). The results of the glass wall washes showed only minimal residues were found (<1% of original amount spiked). Therefore, the losses may be attributed to biotic metabolism by the yeast and/or abiotic degradation from the relatively reductive (anaerobic) environment created by fer-

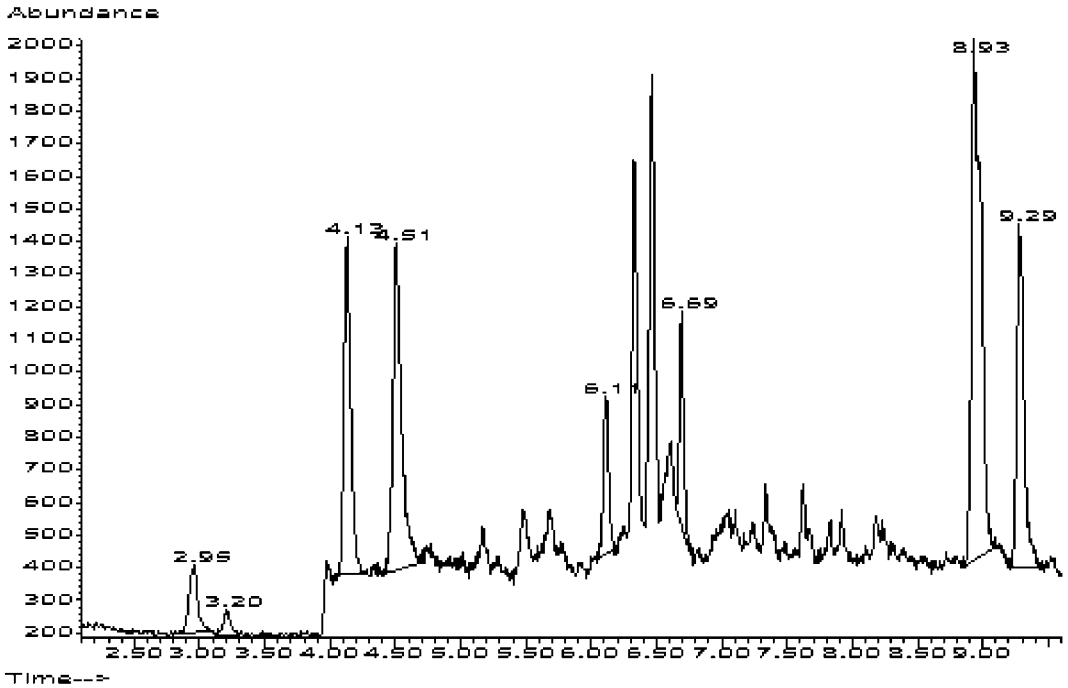

Figure 2. Selected ion chromtograms of 0.0005 ppm recovery in beer. Oxyfluorfen (internal standard, $m/z = 361$, $R_t = 2.95$ min), chlorfenapyr ($m/z = 408$, $R_t = 3.20$ min), quinoxyfen ($m/z = 309$, $R_t = 4.13$ min), tebuconazole ($m/z = 250$, $R_t = 4.51$ min), fenarimol ($m/z = 332$, $R_t = 6.11$ min), pyridaben ($m/z = 366$, $R_t = 6.69$ min), *Z*-dimethomorph ($m/z = 389$, $R_t = 8.93$ min), and *E*-dimethomorph ($m/z = 389$, $R_t = 9.29$ min).

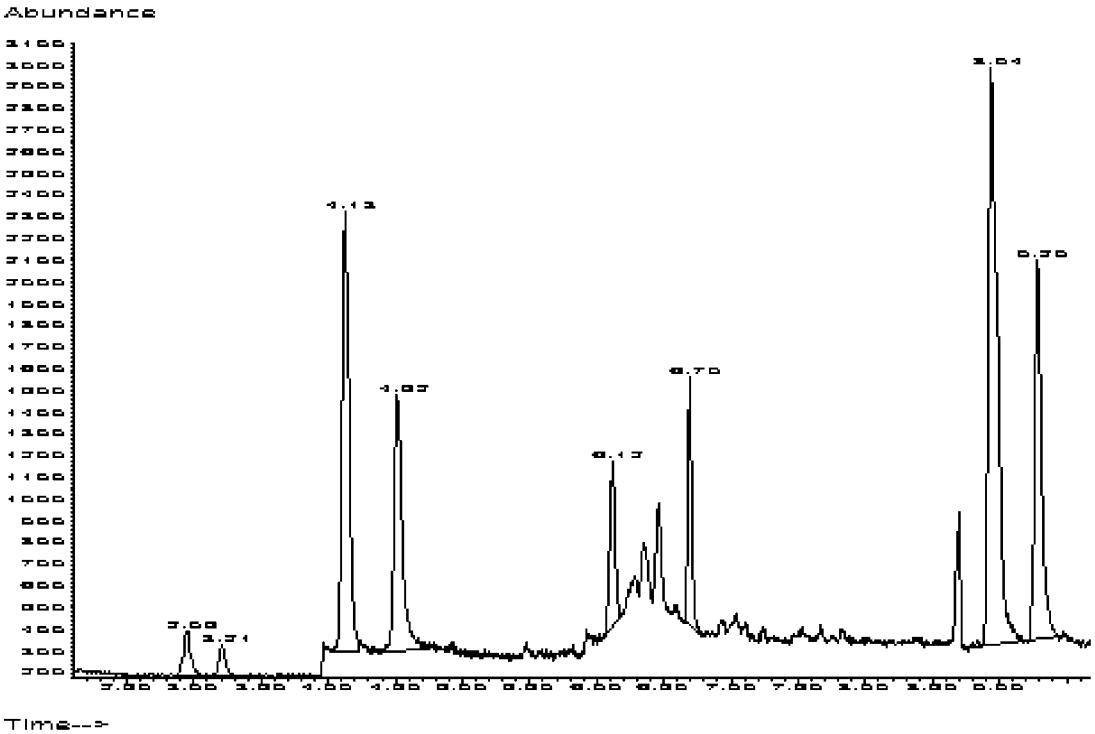

Figure 3. Selected ion chromatograms of 0.5 ppm recovery in hops. Oxyfluorfen (internal standard, $m/z = 361$, $R_t = 2.95$ min), chlorfenapyr ($m/z = 408$, $R_t = 3.21$ min), quinoxyfen ($m/z = 307$, $R_t = 4.13$ min), tebuconazole ($m/z = 250$, $R_t = 4.52$ min), fenarimol ($m/z = 332$, $R_t = 6.12$ min), pyridaben ($m/z = 366$, $R_t = 6.70$ min), *Z*-dimethomorph ($m/z = 389$, $R_t = 8.94$ min), and *E*-dimethomorph ($m/z = 389$, $R_t = 9.29$ min).

mentation (*20*, *21*). Yet another route could be explained by Henry's Law, which suggests that a low volatility and/or low water solubility compound may escape to the atmosphere (*22*, *23*). This process would be greatly facilitated by constant evolution of CO_2 from the fermenting yeast, which would mimic the bubbling of air or nitrogen through a solution to determine the Henry's Law value for a given compound.

Fate of Pesticides in Treated Hops during the Brewing Process. Prior to addition into the brew, the residue levels of the pesticides in the treated hops were determined. Chlorfenapyr was 0.752 ppm, quinoxyfen was 0.245 ppm, tebuconazole was 0.508 ppm, fenarimol was 0.157 ppm, *Z*-dimethomorph was 1.23 ppm, and *E*-dimethomorph was 2.61 ppm. Simple dilution of hops in wort would drop expected residues to approximately

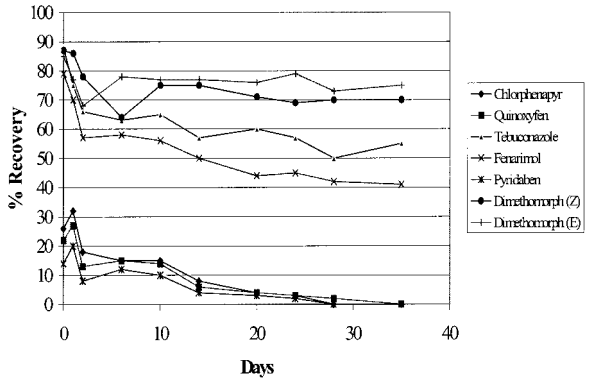

Figure 4. Residue monitoring during fermentation spike experiment. Initial fortification level was 1.0 ppm. Each point represents an average of duplicate experiments.

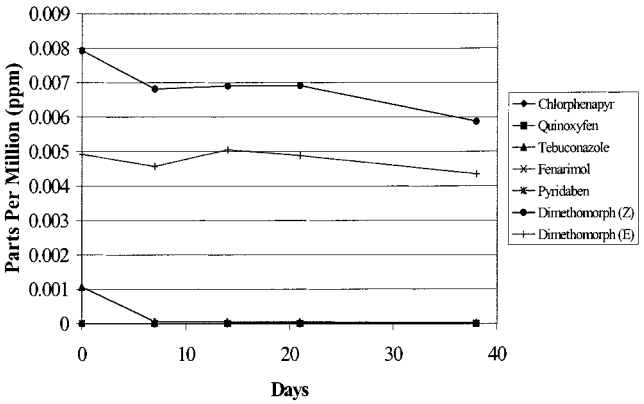

Figure 5. Residue monitoring during fermentation of beer brewed with treated hops. Points represent an average of duplicate experiments.

1% of the original residue concentration on hops. From the brewing trial conducted with field-treated hops, only three compounds carried over into the wort. Tebuconazole, *Z*-dimethomorph, and *E*-dimethomorph were detected at 0.001, 0.008, and 0.005 ppm, respectively. These residues represent less than 31% of the diluted residues. Subsequent analysis of the spent hops showed that 89%, 103%, 100%, 109%, 84%, 103%, and 95% (chlorfenapyr, quinoxyfen, tebuconazole, fenarimol, *Z*-dimethomorph, and *E*-dimethomorph, respectively) of the original residues remained on the spent hops. The high recovery of pesticides in the spent hops can be explained by the highly lipophilic components of hops, which include waxes and resins (*1, 24*). Thus, relatively lipophilic pesticides would have a tendency to remain in/on the hops during the boiling process, and only the compounds with low K_{ow} values would partition, in some small amount, into the wort. Again, a correlation equation was calculated by plotting log K_{ow} (x values) of tebuconazole, *Z*-dimethomorph, *E*-dimethomorph, and pyridaben versus the corresponding percent recovery of residues in the young beer (y values). These points were chosen because residues of tebuconazole, *Z*-dimethomorph, and *E*-dimethomorph were found in the wort, while pyridaben was included as a representative of the upper K_{ow} value in the present study. The resulting equation was $y = -7.935x + 50.3$ ($r^2 = 0.9878$). The observed correlation between hops and wort compares well with the correlation between trub and wort.

Following the addition of yeast to the wort, the carried over residues were monitored for 38 days (**Figure 5**). At the end of monitoring, the tebuconazole residues dropped below the limit of quantitation (0.0005 ppm), whereas *Z* and *E*-dimethomorph residues showed little reduction in the young beer. Analysis of the resulting trub showed only trace (above LOD, but below LOQ) amounts of the carried over residues. These results are consistent with the fermentation results mentioned above.

With the development of the multi-residue method in this study, the fate of seven pesticides was determined in the beer brewing process. This new methodology was shown to be rugged and sensitive for all the various matrixes, and can be easily scaled up for a greater number of compounds in the future. The utilization of mass selective detection provided both quantitation and confirmation of residues.

Pesticide residues found on commercially treated hops were shown to not carry over into the beer at an appreciable level, except for dimethomorph. Even then the level of residue was still very low, given the high level of residues found on the raw commodity. As a result, the potential risk of pesticide exposure from the consumption of beer produced from hops treated with the seven agrochemicals studied is low.

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