JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Fate of Pesticides during Beer Brewing

Tomonori Inoue, Yasushi Nagatomi, Keiko Suga, Atsuo Uyama, and Naoki Mochizuki*

Department of Safety Evaluation, Research Laboratories for Food Safety Chemistry, Asahi Breweries, Ltd., 1-1-21 Midori, Moriya-Shi, Ibaraki 302-0106, Japan

Supporting Information

ABSTRACT: The fates of more than 300 pesticide residues were investigated in the course of beer brewing. Ground malt artificially contaminated with pesticides was brewed via steps such as mashing, boiling, and fermentation. Analytical samples were taken from wort, spent grain, and beer produced at certain key points in the brewing process. The samples were extracted and purified with the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method and were then analyzed by LC-MS/MS using a multiresidue method. In the results, a majority of pesticides showed a reduction in the unhopped wort and were adsorbed onto the spent grain after mashing. In addition, some pesticides diminished during the boiling and fermentation. This suggests that the reduction was caused mainly by adsorption, pyrolysis, and hydrolysis. After the entire process of brewing, the risks of contaminating beer with pesticides were reduced remarkably, and only a few pesticides remained without being removed or resolved.

KEYWORDS: beer, process of brewing, pesticides, residues, LC-MS/MS, QuEChERS

INTRODUCTION

Pesticides play a large role in improving agricultural efficiency, and approximately 1000 pesticides are used in the world. However, a recent trend in rising social requirements for food safety and stricter regulations for pesticides can be seen across the globe. For instance, in Japan, "the Japanese positive list system for agricultural chemical residues in foods" was enforced on May 29, 2006. Food and beverage manufacturers have therefore had to strictly manage the risks of spiked pesticides to comply with these tight regulations and sensitive consumers.

The same goes for breweries. Presently, the safety of manufactured beers and raw materials for beer is guaranteed by self-imposed analysis. Nonetheless, it is essential to elucidate the fate of pesticides during brewing to address the risk of pesticide contamination. Several excellent studies have been reported on the fate of pesticide residues during brewing.[1−8] These systematic studies closely followed the residual tendency of some pesticides in beers. However, since enforcement of the positive list system in Japan, for example, the number of pesticides that need to be monitored has increased dramatically. In addition, recent development of analytical instruments has enabled rapid quantitative analysis of a large number of pesticides by LC-MS/MS using a multiresidue method.[9−11]

This study aims to comprehend the fate of pesticides that can be present during brewing. To obtain exhaustive experimental data, ground malt was artificially contaminated with more than 300 pesticides and was brewed in a laboratory. These pesticides were widely used in growing agricultural products, including barley and hops. The samples were analyzed by LC-MS/MS in certain stages of the brewing process such as mashing, boiling, and fermentation.

MATERIALS AND METHODS

Materials. Pesticide standard solutions, Mix4 (includes 30 pesticides, each 20 ppm in acetonitrile solution), Mix5 (29 pesticides, each 20 ppm in acetnitrile solution), Mix6 (29 pesticides, each 20 ppm in acetonitrile solution), Mix7 (10 pesticides, each 50 ppm in acetonitrile solution), Mix8 (21 pesticides, each 20 ppm in acetonitrile solution), Mix9 (16 pesticides, each 20 ppm in acetonitrile solution), and Mix10 (20 pesticides, each 20 ppm in acetonitrile solution), were purchased from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). Other pesticide standard solutions, Mix22 (includes 50 pesticides, each 10 ppm (except acephate 50 ppm and methamidophos 50 ppm) in acetonitrile solution), Mix31 (85 pesticides, each 10 ppm in acetone/hexane (1/1) solution), Mix34 (46 pesticides, each 10 ppm (except acetamiprid 50 ppm) in acetone solution), Mix48 (61 pesticides, each 10 ppm in acetone/hexane (1/1) solution), and Mix51 (26 pesticides, each 10 ppm in acetone/hexane (1/1) solution), were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The solvents acetonitrile (for pesticide residue and polychlorinated biphenyl (PCB) analytical grade (×5000)), toluene (for pesticide residue and polychlorinated biphenyl (PCB) analytical grade (×5000)), acetone (for pesticide residue and polychlorinated biphenyl (PCB) analytical grade (×5000)), *n*-hexane (for pesticide residue and polychlorinated biphenyl (PCB) analytical grade (×5000)), methanol (for LC-MS), ammonium acetate (JIS-guaranteed reagent) were also purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Triethylamine (JIS-guaranteed reagent) and formic acid (for LC-MS) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

For sample preparation, a Dispersive SPE Citrate Extraction Tube and Dispersive SPE PSA/C18 SPE Cleanup tube1 from Supelco were prepared (Bellefoute, PA). Analytical samples were filtered through a PTFE filter with mesh of 0.2 μm, purchased from Advantec Toyo Kaisha, Ltd. (Tokyo, Japan).

Apparatus. As a liquid chromatography system, an Acquity UPLC equipped with an Acquity BEH C18 column (1.8 μm, 2.0 × 50 mm) (Waters, Milford, MA) was used. Five microliters of each analytical sample was injected into the column with the temperature controlled

Received: November 17, 2010
Revised: February 24, 2011
Accepted: February 24, 2011
Published: March 14, 2011

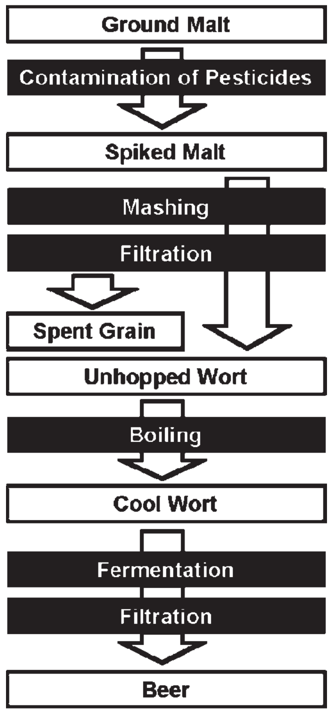

Figure 1. Scheme of principal steps of laboratory-scale brewing.

at 40 °C. Elution was carried out at 0.3 mL/min of a pump flow rate. Mobile phase solvents consisted of two types of eluents: (A) 5 mM ammonium acetate aqueous and (B) 5 mM ammonium acetate in methanol. A linear gradient profile was applied with following proportions: 0 min (95% A, 5% B), 1 min (60% A, 40% B), 2 min (35% A, 65% B), 8 min (95% A, 5% B), 11 min (100% A, 0%), and 11.5 min (95% A, 5% B).

The MS/MS system interfaced with the UPLC unit was an API 4000, a triple-quadrupole tandem mass spectrometer (ABSciex, Foster City, CA). Electrospray ionization (ESI) was performed in positive- or negative-ion mode, depending on the character of each pesticide. In the positive mode, the ion spray voltage was set at 5500 V, the nebulizer gas was at 30 psi, the curtain gas was at 10 psi, and the ion source temperature was set at 500 °C. In the negative mode, the ion spray voltage was set at −4500 V, the nebulizer gas was at 30 psi, the curtain gas was at 20 psi, and the ion source temperature was set at 400 °C. Data for quantification and confirmation were acquired in the multiple reaction monitoring (MRM) mode. The precursor (Q1)-to-fragment (Q3) transitions, the optimum declustering potential (DP), the optimum collision energy (CE), and the optimum collision cell exit potential (CXP) are given for each compound (Table S1 of the Supporting Information).

Laboratory-Scale Brewing from Malt Artificially Spiked with Pesticides. Beer is produced from malt, hops, water, and yeast (some auxiliary materials may be used), among which the malt is the main raw material; when the malt rather than other materials is contaminated with pesticides, the risk of pesticide carry-over to beer becomes highest. Therefore, in this study, the malt was focused on as a subject of contamination.

Each solution that contained different numbers of pesticides (Hayashi Pure Chemical Ind., Ltd., Mix4, Mix5, Mix6, Mix7, Mix8, Mix9, Mix10; Kanto Chemical Co., Inc., Mix22, Mix31, Mix34, Mix48, Mix51) was added to ground malt (110 g) at a concentration of 100 ppb (except for azinphos-methyl, anilofos, aramite, epoxiconazole, oryzalin, carbaryl, carbofuran, silafluofen, diallate, tetrachlorvinphos, pirimicarb, fenobcarb, fenamidone, fluridone, bendiocarb, and methiocarb (200 ppb) and acephate, methamidophos, and acetamiprid (500 ppb)). After the spiked malt was mixed with hot water (200 mL), the first mashing was performed at 55 °C for 60 min. To the mixture were added mashed corn starches and hot water until a total volume of 800 mL was obtained. Further mashing was performed at 65 °C for 60 min and then at 76 °C for 5 min. The mash was filtered to obtain primary wort. Then sparging liquor was added to separately gain secondary wort and spent grain (230 g). The unhopped wort (720 mL) was boiled with hops (approximately 500 mg) in an oil bath at 140 °C for 1 h. The wort was cooled and then filtered to remove hot trub. Yeast (1.5 g) was added to the cooled wort filtrate (300 mL), which was then fermented at 10 °C for 7 days with stirring conducted by a magnetic stirrer and at 10 °C for 7 days and then at 4 °C for 4 days without stirring. After fermentation, the beer was made by removing yeast (Figure 1).

Sample Preparation Method. Sample preparation was carried out by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. This convenient method has been often applied in analyzing pesticide residues.[12−16] Detailed procedures are described as follows: Acetonitrile (10 mL) was added to a sample (10 g) in a 50 mL polypropylene centrifuge tube and was vigorously shaken for 1 min. After addition to a Dispersive SPE Citrate Extraction Tube (Supelco), the mixture was shaken for 1 min and was centrifuged at 3000 rpm for 5 min. After this step, the mixture containing spent grain or the cooled wort was placed in a refrigerator for 1 h so that the lipids separated from the mixture. An aliquot of the extract (6 mL) was transferred to a Dispersive SPE PSA/C18 SPE Clean Up Tube1 (Supelco). The mixture was shaken for 30 s and centrifuged at 3000 rpm for 5 min. An aliquot of the supernatant (4 mL) was transferred to a 15 mL polypropylene centrifuged tube, and 5% (v/v) formic acid in acetonitrile (40 μL) was added to acidify it. The extract (1.5 mL) was filtrated through a PTFE filter with 0.2 μm mesh and was transferred to a 2 mL glass vial.

Method Validation. The experimental method was validated by determining the relative standardard deviation of repeatability (RSD), recovery, and linearity. The repeatability and recovery experiments involved six replicate measurements of each sample. With each test, each blank sample was spiked with the mixture containing pesticides both before and after it was properly prepared at a concentration of 50 ppb (except azinphos-methyl, anilophos, aramite, epoxiconazole, oryzarin, carbaryl, carbofuran, silafluofen, diallate, tetrachlorvinphos, pirimicarb, fenobcarb, fenamidone, fluridone, bendiocarb, and methiocarb (100 ppb) and acephate, metamidphos, and acetamiprid (250 ppb)). Linearity of standard addition calibration curves was estimated at a range from 5 to 100 ppb (except azinphos-methyl, anilophos, aramite, epoxiconazole, oryzarin, carbaryl, carbofuran, silafluofen, diallate, tetrachlorvinphos, pirimicarb, fenobcarb, fenamidone, fluridone, bendiocarb, and methiocarb (from 10 to 200 ppb) and acephate, metamidophos, and acetamiprid (from 25 to 500 ppb)).

Sample Analysis. The residual ratios were calculated on the basis of the concentration of the pesticides present at each step of brewing, compared to that initially spiked to the ground malt. The concentration levels were quantified using standard addition calibration curves at a range of 5−100 ppb (except azinphos-methyl, anilophos, aramite, epoxiconazole, oryzarin, carbaryl, carbofuran, silafluofen, diallate, tetrachlorvinphos, pirimicarb, fenobcarb, fenamidone, fluridone, bendiocarb, and methiocarb (10−200 ppb) and acephate, metamidphos, and acetamiprid (25−500 ppb)). The ratio of initially spiked pesticides was set at 100%.

The Pesticide Manual, 14th ed., was referred to for the properties of pesticides, such as chemical classes, molecular formulas, and log *P* values.[17]

■ RESULTS AND DISCUSSION

Validation Tests. Validation tests were performed on cooled wort (Supporting Information Table S2), spent grain (Supporting Information Table S3), and beer (Supporting Information

Table 1. Residual Ratios of Each Pesticide in Unhopped Wort, Spent Grain, Cooled Wort, and Beer to the Original Residue Concentration before Brewing and Their Classes and Log *P* Values

	pesticide residual ratios					
compound	unhopped wort	spent grain	cooled wort	beer	class	log *P*
1-naphthaleneacetic acid				37.5	biopesticide	2.60
2-(1-naphthyl)acetamide	97.1	41.6	65.6	72.9	synthetic auxin	
2,4-D				33.1	alkylchlorophenoxy	−0.83
4-CPA				47.3	auxin	2.25
abamectin B1a	0.0	106.3	0.0	0.0	biopesticide	4.40
acephate		0.9	46.9	37.1	organophosphate	−0.85
acetamiprid	43.4	21.2	61.5	54.9	neonicotinoid	0.80
acetochlor	15.9	63.0	4.8	8.7	chloroacetamide	4.14
acibenzolar-*S*-methyl	25.5	97.1	10.2	18.7	benzothiadiazole	3.10
acifluorfen	44.1		26.6	4.2	nitrophenyl ether	1.19
acrinathrin	0.0	78.1	0.2	0.0	pyrethroid	5.60
alachlor	5.2	82.8	0.0	12.1	chloroacetamide	3.09
aldicarb	101.3	14.1	0.0	14.4	carbamate	1.36
aldoxycarb/aldicarb sulfone	67.8	5.2	53.2	51.1	carbamate	−0.57
allethrin	0.0	81.4	0.0	0.6	pyrethroid	4.96
ametryn	4.5	87.5	0.0	0.0	triazine	2.63
anilofos	0.0	93.8	0.0	0.0	organophosphate	3.81
aramite	1.5	99.7	0.0	0.7	sulfite ester	4.82
atrazine	19.7	71.9	11.2	11.2	triazine	2.50
azaconazole	51.1	46.4	23.3	11.0	triazole	2.36
azafenidin	16.9	58.1	0.0	8.2	triazolinone	2.70
azamethiphos	60.4	9.8	0.0	7.4	organophosphate	1.05
azimsulfuron				0.0	sulfonylurea	0.04
azinphos-methyl	9.0	84.3	0.0	0.0	organophosphate	2.96
azoxystrobin	13.6	78.4	0.0	9.4	strobilurin	2.50
benalaxyl	0.0	78.9	0.0	0.0	acylalanine	3.54
bendiocarb	54.7	25.5	0.0	0.4	carbamate	1.72
benfluralin	7.8	62.0	0.0	13.2	dinitroaniline	5.29
benoxacor	19.7	53.9	16.3	7.8	benzoxazine	2.69
bensulfulon-methyl	8.5	26.2	0.0	0.0	sulfonylurea	0.79
benzofenap	0.0	93.3	0.0	0.0	benzoylpyrazole	4.69
bifenox		98.4		0.0	diphenyl ether	3.64
bifenthrin	0.0	99.0	0.0	0.0	pyrethroid	7.30
bitertanol	0.0	90.2	0.0	0.2	triazole	4.10
boscalid	0.0	85.4	0.0	0.0	carboxamide	2.96
bromacil	65.8	28.3	48.9	32.6	uracil	1.88
bromobutide	11.7	71.3	0.0	2.6	amide	3.48
bromophos-ethyl				8.6	organothiophosphate	6.15
bromophos-methyl	0.0	60.8	0.4	5.0	organothiophosphate	5.21
bromoxynil	56.3		53.8	15.2	hydroxybenzonitrile	1.04
bupirimate	0.0	75.3	0.0	0.0	pyrimidinol	3.90
buprofezin	0.0	78.3	0.0	0.0		4.80
butachlor	0.0	98.4	0.0	0.0	chloroacetamide	4.50
butafenacil	0.0	93.5	0.0	0.0	uracil	3.20
butamifos	0.0	91.6	0.0	0.0	organophosphate	4.62
butylate		192.6	58.9		thiocarbamate	4.10
cadusafos	10.3	73.1	7.5	2.3	organothiophosphate	3.85
cafenstrole	0.0	68.4	0.0	0.0	triazole	3.21
carbaryl	42.2	51.3	8.0	8.9	carbamate	1.85
carbofuran	72.0	20.7	28.6	24.0	carbamate	1.52
carbofuran-3-hydroxy	135.0	12.6	24.4	41.2	carbamate	1.45

dx.doi.org/10.1021/jf104421q |*J. Agric. Food Chem.* 2011, 59, 3857–3868

Table 1. Continued

compound	pesticide residual ratios: unhopped wort	spent grain	cooled wort	beer	class	log *P*
carboxin	32.5	30.8	23.7	29.7	oxathiin	2.30
carfentrazone-ethyl	0.0	67.8	0.0	0.9	triaolinone	3.36
carpropamid	0.0	91.1	0.0		cyclopropanecarboxamide	4.23
chlorbufam	2.2	99.3	2.0	4.2	carbanilate	3.02
chlorfenapyr	16.7	55.9	35.6		arylpyrrole	4.83
chlorfenvinphos (*E*)		96.2		0.0	organophosphate	4.22
chlorfenvinphos (*Z*)	1.9	95.1	0.0	0.0	organophosphate	3.85
chloridazon	70.3	24.0	31.3	50.6	pyridazinone	1.19
chlorimuron-ethyl			0.0	0.0	sulfonylurea	0.11
chloroxuron	0.0			0.0	dimethylurea	3.40
chlorpropham	15.0	88.7	7.7	0.0	carbanilate	3.79
chlorpyrifos	0.0	61.2	4.8	0.0	organophosphate	4.70
chlorpyrifos-methyl	0.1	69.1	0.2	3.3	organophosphate	4.24
chlorsulfuron			0.0	0.0	sulfonylurea	−0.99
chromafenozide	0.0	89.1	0.0	0.0	oxazolidine	3.30
cinidon-ethyl	4.6	107.8	0.0	0.6	dicarboximide	5.40
cinosulfuron	38.1	5.1	0.0	0.0	sulfonylurea	0.20
clodinafop acid				52.5	aryloxyphenoxypropionate	−0.44
clofentezine		1.2	0.0	0.0	tetrazine	4.10
clomazone	38.6	50.8	20.9	18.3	isoxazolidinone	2.50
clomeprop	0.0	89.8	0.0	0.0	anilide	4.80
cloprop				52.0	chlorophenoxy acid or ester	2.39
cloquintocet-mexyl	0.0	100.6	0.0	0.0		5.03
cloransulam:methyl			37.7	22.6	sulfonanilide	−0.37
clothianidin	52.7		65.1	37.7	neonicotinoid	0.91
cumyluron	0.0	85.2	0.0	0.0	urea	2.61
cyanophos					organophosphate	2.65
cyazofamid			0.0	0.0	cyanoimidazole	3.20
cyclanilid	66.1		43.3	20.7	malonanilate	3.25
cycloate	26.5	119.5	13.9	15.6	thiocarbamate	3.88
cycloprothrin	0.0	91.8	0.0	0.0	pyrethroid	4.19
cyclosulfamuron	0.0	49.5	0.0	0.0	sulfonylurea	1.41
cyflufenamide	0.0	87.8	0.0	0.0	amide	4.70
cyfluthrin	12.0	80.3	5.0	0.0	pyrethroid	6.00
cyhalofop-butyl	0.0	97.1	0.0	0.0	aryloxyphenoxypropionate	3.32
cyhalothrin	0.4	72.6	10.5		pyrethroid	6.80
cyanazine	38.9	49.1	31.4	29.1	triazine	5.30
cypermethrin	1.1	81.0	3.6	10.6	triazole	3.10
cyproconazole	14.5	66.0	12.7	0.0	anilinopyrimidine	4.00
cyprodinil	0.0	95.3	0.0	0.0	phenylurea	2.70
deltamethrin	4.5	57.2	0.0	0.8	pyrethroid	4.60
demeton-*S*-methyl	72.3	9.0	0.0	9.3	organophosphate	1.32
diallate		97.5		14.3	thiocarbamate	3.29
diazinon	3.8	83.6	0.7	0.0	organophosphate	3.69
dichlofenthion	8.2	95.1	21.2	0.7	organothiophosphate	5.14
dichlofluanid	0.0			0.0	sulfamide	3.70
dichlorprop				40.4	aryloxyalkanoic acid	2.29
dichlorvos		42.5	59.7		organophosphate	1.90
diclocymet		97.9		3.1	amide	3.97
diclofop-methyl	0.0	78.9	0.0	0.6	aryloxyphenoxypropionate	4.80
diclomezine		89.6		4.2		2.65
dicloran	32.0	56.3	11.5	9.4	chlorophenyl	2.80
diclosulam	27.4		28.9	23.0	sulfonanilide	0.85

Table 1. Continued

	pesticide residual ratios					
compound	unhopped wort	spent grain	cooled wort	beer	class	log *P*
dicrotophos	128.8	9.6	38.1	52.5	organophosphate	−0.50
diethofencarb	24.0	73.3	23.5	21.5	carbamate	3.02
diflubenzuron	0.0	89.8	0.0	0.0	benzoylurea	3.89
diflufenican	0.0	102.3	0.0	0.9	carboxamide	4.20
dimepiperate	0.0	78.3	0.0	0.0	thiocarbamate	4.02
dimethametryn	0.0	75.3	0.0	0.0	methylthiotriazine	3.90
dimethenamid	35.1	65.8	16.9	28.3	chloroacetamide	2.20
dimethirimol	64.2	50.4	21.5	28.9	pyrimidinol	1.90
dimethoate	110.2	1.8	38.7	46.1	organophosphate	0.70
dimethomorph (*Z*)	11.0	75.9	0.0	14.1	morpholine	2.73
dimethomotph (*E*)	7.6		0.0	14.9	morpholine	2.63
dimethylvinphos (*E*)	1.3	76.8	0.0	0.0	organophosphate	3.13
dimethylvinphos (*Z*)	0.0	82.5	0.0		organophosphate	3.13
dioxathion	0.6	102.6	0.0	4.2	organophosphate	3.45
diphenamid	53.3	46.1	22.8	18.9	alkanamide	2.17
disulfoton		74.0		1.3	organophosphate	3.95
disulfoton-sulfone	84.7	53.9	17.0	31.4		
diuron	19.6	53.0	11.2	13.1	phenylurea	2.85
dymuron	0.0	80.8	0.0	0.0	phenylurea	2.70
edifenphos	0.0	76.2	0.0	0.0	organophosphate	3.83
endosulfan-sulfate	11.0	69.6	5.4	6.7	organochlorine	3.13
endsulfan (α, *β*)	17.2	108.1	9.4	14.8		
EPN	0.0	83.9	0.0	0.0	organophosphate	5.02
epoxiconazole	0.2	115.6	0.0	0.0	triazole	3.30
EPTC					thiocarbamate	3.20
esprocarb	0.0	69.6	4.5	0.0	thiocarbamate	4.60
ethametsulfuron-methyl	31.0		0.0	0.0	triazinylsulfonylurea	0.89
ethiofencarb	39.8	26.2	31.2	28.3	carbamate	2.04
ethion	0.0	84.4	0.0	1.3	organophosphate	5.07
ethofumesate	20.9	68.9	8.4	6.7	benzofuran	2.70
ethoprophos	27.8	72.2	5.4	12.1	organophosphate	2.99
ethoxysulfuron	24.0		0.0	0.0	sulfonylurea	1.01
etofenprox	0.0	100.4	0.0	0.0	pyrethroid	6.90
etoxazole	0.0	89.4	0.0	0.0	diphenyloxazoline	5.52
etrimfos	3.0	84.1	8.0	0.0	organothiophosphate	2.94
fenamidone	0.0	96.4	0.0	0.0	imidazole	2.80
fenamiphos	11.2	61.6	0.0	0.0	organophosphate	3.30
fenarimol	0.0	83.9	0.0	0.7	pyrimidine	3.69
fenbuconazole	0.0	70.9	0.5	0.0	pyrimidine	3.79
fenhexamid	0.0	86.3	5.7	4.3	hydroxyanilide	3.51
fenobucarb	36.7	62.3	27.4	21.0	carbamate	2.78
fenothiocarb	4.7	75.7	0.2	2.7	thiocarbamate	3.28
fenoxanil	0.0	89.1	0.0	0.0	amide	3.53
fenoxaprop-ethyl	0.0	90.2	1.3	0.0	aryloxyphenoxypropionate	4.28
fenoxycarb	0.0	96.4	0.0	0.0	juvenile hormone mimic	4.07
fenpropathrin	0.0	102.1	0.0	1.9	pyrethroid	6.04
fenpropimorph	1.9	77.0	0.7	0.9	morpholine	4.50
fenpyroximate (*E*)	0.0	110.0	0.0	0.0	pyrazole	5.01
fenpyroximate (*Z*)	0.0		0.0	0.0	pyrazole	5.01
fensulfothion	33.1	54.1	17.7	11.3	organophosphate	2.23
fenthion	0.0	43.1	4.7	0.0	organophosphate	4.84
fenvalerate	4.7	59.0	0.0	0.9	pyrethroid	5.01
ferimzone	17.6	71.1	0.0	0.0	pyrimidine	2.89

Table 1. Continued

	pesticide residual ratios					
compound	unhopped wort	spent grain	cooled wort	beer	class	log *P*
fipronil		94.9		8.1	phenylpyrazole	3.75
flamprop-methyl	8.6	75.3	0.3	1.0	aryaminopropionic acid	2.90
flazasulfuron	23.9	1.3	0.0	0.0	sulfonylurea	−0.06
florasuram	60.1		66.2	45.5	triazolopyrimidine	−1.22
fluacrypyrim	0.0	78.9	0.0	0.0	strobilurin	4.50
fluazifop				57.7	aryloxyphenoxypropionate	3.18
flucythrinate	0.4	98.6	0.0	0.0	pyrethroid	4.74
fludioxonil		104.3		0.0	phenylpyrrole	4.12
flufenacet			0.0	0.0	oxyacetamide	3.20
flufenoxuron	0.0	92.0	0.0	2.6	benzoylurea	4.01
flufenpyr-ethyl		95.3		0.0	pyridazinone	
flumetsulam	60.6		68.0	67.2	triazolepyrimidine	0.21
flumiclorac-pentyl	0.0	73.7	2.1	2.7	dicarboximide	4.99
flumioxazin			4.4		dicarboximide	2.55
fluoroxypyr				67.2	pyridine compound	2.00
fluquinconazole	0.0	96.0	0.0	5.1	triazole	3.24
fluridone	12.7	80.8	0.0	0.0		1.87
flusilazole	0.0	94.0	0.0	0.0	triazole	3.87
fluthiacet-methyl	0.0	62.7	0.0	0.0		3.77
flutolanil	0.0	91.8	0.0	0.6	oxathiin	3.17
flutriafol		47.9		8.4	triazole	2.30
fluvalinate	2.1	97.9	0.0	0.0	pyrethroid	4.26
fomesafen	32.6		23.6	2.4	diphenyl ether	−1.20
foramsulfuron				0.0	pyrimidinylsulfonylurea	−0.78
forchlorfenuron	2.2		1.8	6.2	phenylurea	3.20
formothion	5.6	0.0	0.0	0.0	organothiophosphate	1.48
fosthiazate	80.6	18.4	38.0	26.9	organophosphate	1.68
furametpyr	52.8	37.2	7.0	24.1	carboxamide	2.36
furathiocarb	0.0	34.3	0.0	0.0	carbamate	4.60
gibberellic acid				4.4		
halfenprox	0.0	74.4	2.3	0.0	pyrethroid	7.70
halosulfuron-methyl	10.9	23.6	0.0	0.0	pyrazole	−0.02
haloxyfop	55.9		58.9	35.1	aryloxyphenoxypropionic acid	4.33
hexaconazole	0.5	99.3	3.0	2.9	triazole	3.90
hexaflumuron	0.0	94.6	0.0	0.0	benzoylurea	5.68
hexazinone	84.8	16.6	51.3	35.5	triazinone	1.17
hexythiazox	0.0	92.4	0.0	0.0	carboxamide	2.67
imazalil	0.0	74.0	0.0	0.0	imidazole	2.56
imazamethabenz-methyl ester	79.9	18.0	40.7	28.4	imidazolinone	1.54
imazaquin				67.2	imidazolinone	−1.09
imazosulfuron	0.0	34.3	0.0	0.0	sulfonylurea	1.72
imibenconazole	0.0	73.3	0.0	0.0	triazole	4.94
imibenconazol-desbenzyl	75.8	37.2	61.5	63.9		
imidacloprid	74.4	22.0	59.7	62.0	neonicotinoid	0.57
indanofan	7.8		0.0	0.2		3.59
indoxacarb	0.0	89.4	0.0	0.0	oxadiazine	4.65
iodosulfuron-methyl		32.4		0.0	sulfonylurea	1.59
ioxynil	26.8		5.3	0.0	hydroxybenzonitrile	2.20
iprobenfos	9.6	74.0	0.0	0.5	organophosphate	3.37
iprodione	7.1	70.4	7.4	0.0	dicarboximide	3.10
iprovalicarb		75.5	0.0	0.8	carbamate	3.18
isazofos	0.0	67.0	0.0	0.0	organothiophosphate	3.10
isofenphos	0.0	86.3	0.0	0.0	organophosphate	4.04

Table 1. Continued

	pesticide residual ratios					
compound	unhopped wort	spent grain	cooled wort	beer	class	log *P*
isofenphos-oxon	28.9	62.3	10.8	15.7		
isoprocarb	55.9	48.2	46.4	40.2	carbamate	2.32
isoprothiolane	2.4	73.0	0.0	0.0	phosphorothiolate	3.30
isoxaflutole	0.0	0.0	0.0	0.0	isoxazole	2.32
isoxathion	0.0	62.0	0.0	0.0	organophosphate	3.88
isoxathion-oxon	0.0	3.0	0.0	0.0		
kresoxim:methyl		96.0		5.6	strobilurin	3.40
lactofen	3.1	86.7	0.0	3.3	diphenyl ether	
lenacil	44.0	52.6	40.5	35.4	uracil	1.69
linuron	5.4	78.6	0.0	0.0	urea	3.00
lufenuron	0.0	99.5	0.0	0.0	benzoylurea	5.12
malathion	2.0	81.7	0.0	0.0	organophosphate	2.75
MCPA				36.2	aryloxyalkanoic acid	−0.81
MCPB				11.3	aryloxyalkanoic acid	1.32
mecarbam	7.2	101.5	0.0	1.8	organophosphate	2.29
mecoprop				41.3	aryloxyalkanoic acid	−0.19
mefenacet		85.4	0.0	0.0	oxyacetamide	3.23
mefenpyr-diethyl	0.0	98.8	0.0	0.0		3.83
mepanipyrim	0.0	103.0	0.0	0.0	anilinopyrimidine	3.28
mepronil	0.0	86.5	0.0	0.0	oxathiin	3.66
mesosulfuron-methyl		8.4	0.0	0.0	sulfonylurea	−0.48
metalaxyl	76.2	20.1	45.2	38.5	phenylamide	1.65
methabenzthiazuron		61.8	10.5	17.3	urea	2.64
methamidophos	95.8	3.4	92.3	80.4	organophosphate	−0.79
methidathion	27.3	64.7	0.0	0.0	organophosphate	2.57
methiocarb	7.9	65.8	0.0	0.0	carbamate	3.08
methomyl	63.0	3.6	0.0	7.2	carbamate	0.09
methoprene	8.3	88.0	0.0	0.0	terpene	6.00
methoxyfenozide	18.1	81.4	0.0	3.4	diacylhydrazine	3.72
metolachlor	9.1	77.7	0.0	4.8	chloroacetamide	3.40
metominostrobin (*E*)	56.7	43.9	25.4	18.4	strobilurin	2.32
metominostrobin (*Z*)	73.8	33.4	33.9	29.6	strobilurin	2.32
metosulam	0.0	75.5	11.1	8.1	triazolopyrimidine	0.20
metsulfuron-methyl	46.0	4.6	0.0	0.0	sulfonylurea	−1.70
mevinphos (*E*)	88.9	7.9	27.5	29.9	organophosphate	0.13
mevinphos (*Z*)	95.1	7.5	43.5	44.7	organophosphate	0.13
monocrotophos	84.5	8.9	23.1	20.8	organophosphate	−0.22
monolinuron	43.9	44.2	5.2	11.3	urea	2.20
myclobutanil	7.0	75.3	7.5	0.6	triazole	2.89
naproanilide	0.0	92.4	0.0	0.0	anilide	3.30
napropamide	11.2	61.7	0.0	0.0	alkanamide	3.30
naptalam				1.4	phthalamate	0.00
norflurazon	44.4	49.4	23.8	17.9	pyridazinone	2.45
novalron	3.1	79.5	0.0	5.8	benzoylurea	4.30
oryzalin	20.8	69.3	15.9	10.3	dinitroaniline	3.73
oxadiazon	0.0	77.6	0.0	0.0	oxidiazole	5.33
oxadixyl	97.7	16.9	57.0	41.4	phenylamide	0.65
oxamyl	14.1	3.5	0.0	0.0	carbamate	−0.44
oxaziclomefone	0.0	95.1	0.0	0.0		5.15
oxycarboxine	49.5	14.1	0.0	11.1	oxathiin	0.77
paclobutrazol	13.0	74.0	10.6	8.1	triazole	3.11
parathion	0.0	98.8	0.0	0.0	organophosphate	3.83

Table 1. Continued

compound	pesticide residual ratios				class	log *P*
	unhopped wort	spent grain	cooled wort	beer		
parathion-methyl		77.0			organophosphate	3.00
penconazole		96.8		0.0	triazole	3.72
pencycuron	0.0	40.5	0.0	0.0	phenylurea	4.68
pendimethalin	0.0	63.4	5.3	0.0	dinitroaniline	5.20
penoxsulam			55.3	36.0	triazopyrimidine sulfonamide	−0.60
pentoxazone	0.0	86.5	0.0	0.0		4.66
permethrin	0.0	69.6	0.3	0.0	pyrethroid	6.10
phenmedipham	0.0	79.5	0.0	0.0	bis-carbamate	3.59
phenothrin	0.0	62.3	0.0	0.0	pyrethroid	6.01
phenthoate	0.0	79.9	0.0	0.0	organophosphate	3.69
phorate		97.1		15.3	organophosphate	3.86
phosalone	0.2	91.1	1.7	0.0	organophosphate	4.01
phosmet	9.3	61.9	0.0	0.4	organophosphate	2.96
phosphamidon	86.7	9.3	14.5	11.9	organophosphate	0.80
picolinafen	0.0	98.4	0.0	0.0	pyridine compound	5.43
piperonyl butoxide		105.9		0.0	performance enhancer	4.75
piperophos	0.0	77.1	0.0	0.0	organophosphate	4.30
pirimicarb	82.0	16.3	53.8	38.7	carbamate	1.70
pirimiphos-methyl	1.5	92.7	0.0	1.2	organophosphate	4.08
pretilachlor	0.0	97.9	0.0	0.0	chloroacetamide	4.08
primisulfuron-methyl	15.2	39.0	0.0	0.7	sulfonylurea	0.20
procymidone				0.0	dicarboximide	3.30
prohydrojasmon	0.8	116.7	4.7	8.7		
prometryn	0.0	72.9	0.0	0.0	triazine	3.34
propachlor	47.5	34.7	17.9	11.0	chloroacetamide	1.60
propanil	16.2	66.1	9.9	7.5	anilide	2.29
propaphos	2.4	84.5	0.0	0.0		
propaquizafop	0.0	86.3	0.0	0.0	aryloxyphenoxypropionate	4.78
propargite	0.0	67.8	0.0	0.4	sulfite ester	5.70
propazine	0.0	115.6	0.0	3.1	triazine	3.95
propiconazole	0.0	91.6	0.0	0.0	triazole	3.72
propoxur	91.9	21.7	37.8	31.5	carbamate	0.14
propoxycarbazone-sodium	47.6	24.4	10.6	0.0	triazolone	−1.55
propyzamide	19.1	71.7	5.6	5.7	benzamide	3.30
prosulfuron	22.8	26.0	0.0	0.0	sulfonylurea	1.50
prothiophos	0.3	64.7	0.0	0.0	organophosphate	5.67
pyraclofos	0.0	75.7	0.0	0.0	organophosphate	3.77
pyraclostrobin	0.0	85.6	0.0	0.0	strobilurin	3.99
pyraflufen-ethyl		93.1		0.0	phenylpyrazole	3.49
pyrazolynate		20.7	0.0	0.0	pyrazole	2.58
pyrazophos	0.0	67.1	0.0	0.0	phosphorothiolate	3.80
pyrazosulfuron-ethyl	23.8	15.0	0.0	0.0	pyrazole	3.16
pyributycarb	0.0	103.0	0.0	0.0	thiocarbamate	
pyridaben	0.0	63.4	0.0	0.0	pyridazinone	6.37
pyridafenthion	0.0	72.6	0.0	0.0	organophosphate	3.20
pyrifenox	5.9	77.9	0.0	0.0	pyridine	3.40
pyriftalid		71.3	0.0	0.0		2.60
pyrimethanil	0.2	107.6	0.0	0.0	anilinopyrimidine	2.84
pyrimidifen	0.0	99.0	0.0	0.0		4.59
pyriminobac-methyl (*E*)	11.4	81.4	0.0	8.3	pyrimidinyloxybenzoic	2.51
pyriminobac-methyl (*Z*)	48.8	58.5	51.9	43.0	pyrimidinyloxybenzoic	2.11
pyriproxyfen	0.0	72.6	0.0	0.0	juvenile hormone mimic	5.37

Table 1. Continued

compound	pesticide residual ratios: unhopped wort	spent grain	cooled wort	beer	class	log *P*
pyroquilon	68.1	28.8	56.7	59.6		1.57
quinalphos	0.0	95.7	0.0	0.0	organophosphate	4.44
quinoclamin	43.2	44.9	23.0	6.7		1.58
quinoxyfen	0.0	78.0	0.0	2.9	quinoline	4.66
quizalofop-ethyl	0.0	87.8	0.0	0.0	aryloxyphenoxypropionate	4.28
resmethrin	7.2	121.5	0.0	3.0	pyrethroid	5.43
silafluofen	0.0	74.6	3.6	0.0	pyrethroid	8.20
simazine	49.6	50.4	24.7	20.8	triazine	2.30
simeconazole	21.2		0.0	1.8	conazole	3.20
simetryn	20.4	96.4	3.7	13.8	triazine	2.80
spinosyn A	0.0	98.6	0.0	0.0	spynosyn	4.00
spinosyn D		91.6	0.0	0.0	spynosyn	4.50
spirodiclofen	0.0	52.4	0.0	1.0	tetronic acid	5.83
spiroxamin	2.8	95.7	0.0	0.0	morpholine	2.89
sulfentrazone	65.7	30.6	62.7	47.3	aryl triazolinone	1.48
sulfosulfuron	15.8		0.0	0.0	sulfonylurea	−0.77
TCMTB	0.0	0.0	0.0	3.4	benzothiazole	3.12
tebuconazole	0.0	80.1	0.0	0.0	triazole	3.70
tebufenozide	0.0		0.0		diacylhydrazine	4.25
tebufenpyrad	0.0	77.0	0.0	0.0	pyrazole	4.93
tebuthiuron	104.0	18.9	53.1	61.0	urea	1.79
teflubenzuron	8.3	111.4	0.0	0.2	benzoylurea	4.30
terbacil					uracil	1.89
terbufos		85.4		9.6	organophosphate	4.51
terbutryn	0.0	96.0	0.0	0.0	triazine	3.65
tetrachlorvinphos	0.0	56.5	0.0	0.6	organophosphate	3.53
tetraconazole		102.3		5.8	triazole	3.56
thiabendazole	37.8	59.9	0.0	12.9	benzimidazole	2.39
thiacloprid		37.0	30.6	32.5	neonicotinoid	1.26
thiamethoxam	52.0	22.0	57.1	66.2	neonicotinoid	−0.13
thidiazuron	14.3	74.0	16.7	14.2	phenylurea	1.77
thifensulfuron-methyl	39.3		0.0	0.0	sulfonylurea	−1.70
thifluzamide	0.0	109.4	0.0	0.0	carboxamide	4.16
thiobencarb	1.1	77.9	4.8	0.0	thiocarbamate	4.23
thiodicarb			0.0	0.0	carbamate	1.62
tolclofos-methyl	7.7	93.8	0.0	0.0	chlorophenyl	4.56
tralkoxydim 1	0.0	76.2	0.0	0.0	cyclohexanedione oxime	2.10
triadimefon	8.6	78.1	4.5	4.4	triazole	3.18
triadimenol	17.4	75.9	10.6	14.8	triazole	3.18
triallate	5.3	73.5	5.4	4.2	thiocarbamate	4.06
triasulfuron			0.0	0.0	sulfonylurea	−0.59
triazophos	0.0	119.7	0.0	0.0	organophosphate	3.55
tribenuron-methyl	0.0	0.0	0.0	0.0	sulfonylurea	0.78
terbufos	0.0		0.0	0.0	organophosphate	5.52
triclopyr				48.7	pyridine compound	−0.45
tricyclazole	48.2	41.4	34.7	24.6	triazolobenzothiazole	1.42
tridemorph 2	6.0	84.7	0.0	3.7	morpholine	4.20
trifloxystrobin	0.0	74.4	0.0	0.0	strobilurin	4.50
trifloxysulfuron-sodium			0.0	0.0	sulfonylurea	−0.42
triflumuron	1.8	111.4	0.0	1.0	benzoylurea	4.91

Table 1. Continued

compound	pesticide residual ratios				class	log *P*
	unhopped wort	spent grain	cooled wort	beer		
triticonazole	8.4	74.2	0.0	0.0	triazole	3.29
uniconazole	0.0	61.4	6.2	5.5	triazole	3.84
XMC	63.1	38.1	27.1	22.3	carbamate	2.23
zoxamide				0.4	benzamide	3.76

Table S4) for each pesticide. Criteria for the validation were defined such that the RSD was within 20%, recovery was between 60 and 120%, and linearity (*r*) was >0.98, with reference to the European Union guidelines.[18] In the results, 324 pesticides in cooled wort, 312 pesticides in spent grain, and 356 pesticides in beer satisfied the criteria. It was assumed that the reason for the largest number of pesticides passing the validation tests being in beer was that the contents of the matrix decreased in the beer after fermentation. Matrices can cause ionization suppression in mass spectrometry, called "matrix effects."[19−21]

Behavior Measurement of Pesticides after Brewing. The residual ratios of pesticides in beer made from pesticide-spiked malt were calculated as described under Materials and Methods; 312 pesticides were not detected at all or were detected at trace levels (<5 ppb) in the beer. The only pesticide for which the residual ratio exceeded 80% was methamidophos, and 16 other pesticides remained at >50%.

This result indicated that a majority of the pesticides initially added to the malt were reduced in content during the brewing process of beer.

Behavior Measurement of Pesticide at Each Step of Brewing. It is essential for close risk management to elucidate the detailed fates of pesticides during the entire process of brewing. Their residual ratios in unhopped wort, spent grain, and cooled wort, all of which are produced at key steps in brewing, mashing and boiling, were analyzed (Tables 1 and 2).

First, the residual ratios of pesticides in unhopped wort, which separated from the spent grain after mashing, were observed. Even at this early step, the ratios of 233 pesticides were <30%. Conversely, only 16 remained at >80%. This demonstrated that most of pesticides were reduced in content after mashing. The reason for the reduction was speculated to be that the pesticides were pyrolyzed, hydrolyzed, and adsorbed onto insoluble components.

Next, the residual ratios in spent grain were analyzed; 124 of 312 validated pesticides remained at >80%. When the limit was set at >50%, 112 more pesticides were present. Therefore, the reduction of pesticide residues in the wort was considered to be due to their adsorption onto spent grain during the mashing process.

Then, wort was analyzed after being boiled at 140 °C and cooled to room temperature. Only one pesticide remained at >80% in the cooled wort. This result showed that the residual ratios of some pesticides were reduced during the boiling process. Pyrolysis was thus considered one factor for this reduction. For instance, aldicarb, which diminished significantly during this step, was known to decompose at >100 °C.[22]

Features of Pesticide Adsorption onto Spent Grain. The above results indicated that the reduction of pesticide residues in brewing was mainly due to their adsorption onto spent grain. Therefore, ascertaining the features of pesticides detected in the spent grain would contribute to managing the risk of residual pesticides in manufacturing.

Table 2. Number of Pesticides Remaining in Each Percent Class in Unhopped Wort, Spent Grain, Cooled Wort, and Beer

residue (%)	no. of pesticides			
	unhopped wort	spent grain	cooled wort	beer
80	16	124	1	1
50−80	29	112	21	16
30−50	27	27	20	27
10−30	47	26	41	51
0−10	186	23	241	261
total	305	312	324	356
validation failure	63	56	44	12

Here, the log *P* (partition coefficient) value of each pesticide was focused on, as a measure of the hydrophobic properties of chemical substances. Pesticides having a high log *P* were hydrophobic, and those having a low log *P* were hydrophilic. The log *P* values of many pesticides are published.[17] The correlation between adsorption ratios and log *P* values was plotted on a graph (Figure 2), and it shows pesticides with a higher log *P* tended to be adsorbed more readily onto spent grain. Inversely, on the same chart for unhopped wort, pesticides with a lower log *P* tended to remain in the wort. Pesticides that remained in the wort at >80% especially had log *P* values of <2.

These results suggest that whether pesticides were adsorbed onto spent grain or remained in the unhopped wort correlated with the degree of their log *P*.

Features of Compounds That Dominantly Remained in Beer. Only a few pesticides remained at large ratios in beer after the artificially spiked malt was brewed (Figure 3). In particular, methamidophos remained at about 80%, 2-(1-naphthyl)acetamide and imazaquin remained at 70−80%, and fluoroxypyr, flumetsulam, thiamethoxam, imibenconazole-desbenzyl, imidacloprid, and tebuthiuron remained at 60−70% (Figure 4; Table 3). In terms of their physical properties, these nine pesticides that had low log *P* values, being <2, largely remained in unhopped wort. Hence, special care should be taken with these nine pesticides and their use on raw materials, especially on malt for beer manufacturing.

Risk Prediction Based on Chemical Features. Predicting the residual ratios of pesticides spiked on raw materials is helpful for risk management in beer production. Log *P* values can provide a clue to the prediction. The actual data of this study showed only the pesticides with low log *P* values (<2) remained in beer. If one pesticide with an unconfirmed fate in brewing were detected in the raw material, its log *P* value can help with predicting its residual ratio in brewed beer.

Meanwhile, pesticides are classified according to their chemical structures and are sometimes discussed in each group. In this study, whether pesticides in the same group showed the same behavior during brewing was ascertained. The pesticides in some groups then showed the same behavior, whereas those in other groups did not. Pesticides belonging to the benzoylurea group (e.g., hexaflumuron and teflubenzuron) or the pyrethroid group (e.g., allethrin and silalfluofen) mainly adsorbed onto spent grain due to their hydrophobic properties with high log *P* values. On the other hand, the neonicotinoid group (e.g., thiacloprid and thiamethoxam), which was hydrophilic with low log *P* values, scarcely adsorbed onto spent grain and remained even in fermented beer. Although pesticides of the sulfonylurea group (e.g., cinosulfuron and primisulfuron-methyl) were hydrophilic, they disappeared completely after the wort was boiled, and this result indicated that they decomposed in heat. Unlike these groups, pesticides such as those in the carbamate group (e.g., aldoxycarb and iprovalicarb) or the organophosphate group (e.g., mevinphos and chlorpyrifos) had various log *P* values and chemical stabilities even within the same group. Therefore, they did not show similar behaviors in brewing (Figure 5).

As seen above, some particular chemical groups showed a similarity in their behavior in brewing, whereas other groups did not. This means that comprehensive risk management based only on chemical class is not reasonable.

The detailed analysis in this study elucidated the behavior of residual pesticides in each step of brewing with malt spiked with more than 300 pesticides. In conclusion, most of the pesticides had reduced residual ratios after brewing and were not detected in the resulting beer. Only a part of those pesticides with low log *P* values having hydrophilic properties had the possibility of remaining in the unhopped wort. Such a decrease in the residual level was mainly due to their adsorption onto spent grain.

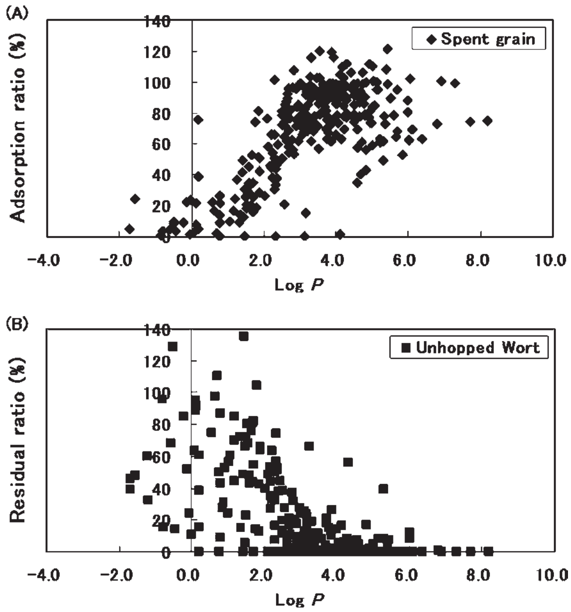

Figure 2. Correlation charts between log *P* value and adsorption ratio on spent grain (A) and between log *P* value and residual ratio in unhopped wort (B).

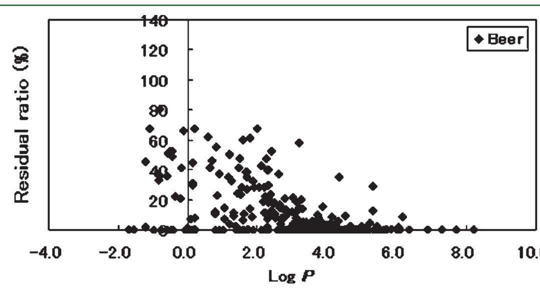

Figure 3. Correlation chart between log *P* value and residual ratio in beer. Only nine pesticides with lower log *P* values (<2) remained at >60%.

Table 3. Pesticides Remaining at >60% in Beer and Their Residual Ratios in Beer, Classes, and Log *P* Values

pesticide	residual ratio (%)	class	log *P*
methamidophos	80.4	organophosphate	−0.79
2-(1-naphtyl)acetamide	72.9	synthesis auxin	
imazaquin	67.2	imidazoline	−1.09
fluoroxypyr	67.2	pyridine	−1.24
flumetsulam	67.2	triazolopyrimidine	0.21
thiamethoxam	66.2	neonicotinoid	−0.13
imibenconazole-desbenzyl	63.9	triazole	
imidacloprid	62.0	neonicotinoid	0.57
tebuthiuron	61.0	urea	1.79

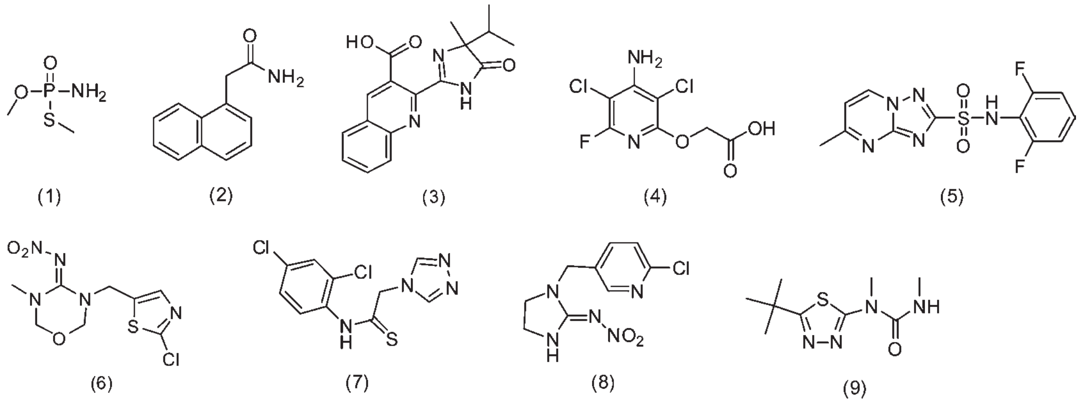

Figure 4. Structures of pesticides remaining at >60% in beer: (1) methamidophos, (2) 2-(1-naphthyl)acetamide, (3) imazaquin, (4) fluoroxypyr, (5) flumetsulam, (6) thiamethoxam, (7) imibenconazole-desbenzyl, (8) imidacloprid, and (9) tebuthiuron.

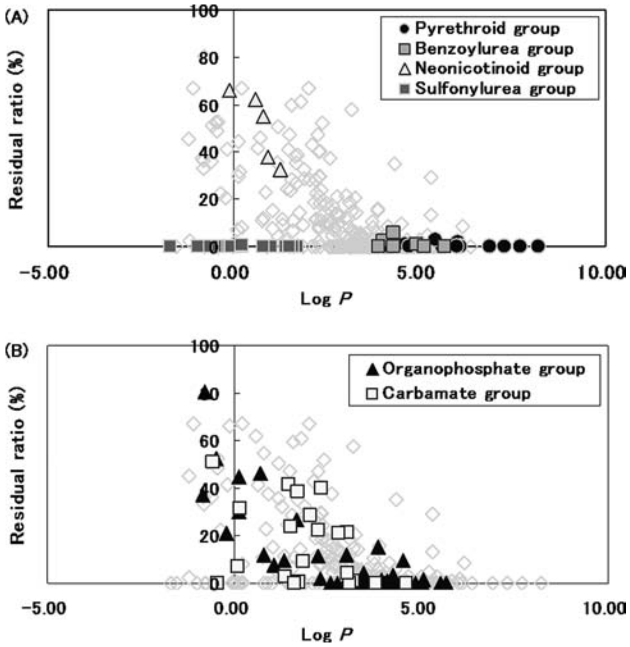

Figure 5. Correlation charts between log *P* value and residual ratio in beer. Pesticides belonging to some groups such as the benzoylurea, pyrethroid, neonicotinoid, or sulfonylurea groups showed similar behaviors (A), whereas pesticides in other groups such as cabamate or organophosphate groups did not (B).

Furthermore, boiling and metabolization by fermentation reduced pesticides' contamination risk in beer.

Having actual data on the fates of pesticides in brewing is very important for managing risk in beer production. These data, residual ratios and relationships with each log *P* value, will make it possible to predict the risk of unexpected pesticides in beer.

ASSOCIATED CONTENT

Supporting Information. Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: naoki.mochizuki@asahibeer.co.jp. Phone: +81-297-46-1827. Fax: +81-297-46-1506.

ACKNOWLEDGMENT

We thank T. Kishimoto for his technical support in beer brewing and M. Yanagisawa for her technical assistance in conducting LC-MS/MS analyses.

REFERENCES

(1) Navarro, S.; Perez, G.; Navarro, G.; Mena, L.; Vela, N. Decay of dinitroanilin herbicides and organophosphorus insecticides during brewing of lager beer. *J. Food Prot.* **2006**, *69*, 1699–1706.

(2) Navarro, S.; Perez, G.; Vela, N.; Mena, L.; Navarro, G. Behavior of myclobutanil, propiconazole, and nuarimol residues during lager beer brewing. *J. Agric. Food Chem.* **2005**, *53*, 8572–8579.

(3) Miyake, Y.; Tajima, R. Fate of pesticide metabolites on malt during brewing. *J. Am. Brew. Chem.* **2003**, *61*, 33–36.

(4) Hengel, M. J.; Shibamoto, T. Method development and fate determination of pesticide-treated hops and their subsequent usage in the production of beer. *J. Agric. Food Chem.* **2002**, *50*, 3412–3418.

(5) Miyake, Y.; Koji, K.; Matsuki, H.; Tajima, R.; Ono, M.; Mine, T. Fate of agrochemical residues, associated with malt and hops, during brewing. *J. Am. Soc. Brew. Chem.* **1999**, *57*, 46–54.

(6) Boivin, P. Official qualification of pesticides used on malting barley. *BIOS Boissons Cond.* **1998**, *29*, 93–97.

(7) Hack, M.; Nitz, S.; Parlar, H. Behavior of [^{14}C]atrazine, [^{14}C]terbutylazine, and their major metabolites in the brewing process. *J. Agric. Food Chem.* **1997**, *45*, 1375–1380.

(8) Nitz, S.; Moza, P. N.; Kokabi, J.; Freitag, D.; Behechti, A.; Korte, F. Fate of ethylenebis(dithiocarbamates) and their metabolites during the brew process. *J. Agric. Food Chem.* **1984**, *32*, 600–603.

(9) Soler, C.; Manes, J.; Pico, Y. The role of the liquid chromatography—mass spectrometry in pesticide residue determination in food. *Crit. Rev. Anal. Chem.* **2008**, *38*, 93–117.

(10) Omote, M.; Harayama, K.; Sasaki, T.; Mochizuki, N.; Yamashita, H. Analysis of simultaneous screening for 277 pesticides in malt and beer by liquid chromatography with tandem mass spectroscopy. *J. Am. Soc. Brew. Chem.* **2006**, *64*, 139–150.

(11) Nunez, O.; Moyano, E.; Galceran, M. T. LC-MS/MS analysis of organic toxics in food. *Trends Anal. Chem.* **2005**, *24*, 683–703.

(12) Lehotay, S. J. Determination of pesticide residues in food by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *J. AOAC Int.* **2007**, *90*, 485–520.

(13) Payá, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the quick easy cheap effective rugged and safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal. Bioanal. Chem.* **2007**, *389*, 1697–1714.

(14) Anastassiades, M.; Lehotay, S. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431.

(15) Guilbault, G. G.; Hjelm, M. Nomenclature for automated and mechanized analysis. *Pure Appl. Chem.* **1989**, *61*, 1657–1664.

(16) Zhang, K.; Wong, J. W.; Hayward, D. G.; Sheladia, P.; Krynitsky, A. J.; Schenck, F. J.; Webster, M. G.; Ammann, J. A.; Ebeler, S. E. Multiresidue pesticide analysis fo wines by dispersive solid-phase extraction and ultrahigh-performance liquid chromatography—tandem mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 4019–4029.

(17) Tomlin, C. D. S., Ed. *The Pesticide Manual*, 14th ed.; British Crop Protection Council: Hampshire, U.K., 2006.

(18) Document No. SANCO/10684/2009. Method validation and quality control procedures pesticide residue analysis in food and feed, 2010; http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf.

(19) Cappiello, A.; Famiglini, G.; Palma, P.; Trufelli, H. Matrix effects in liquid chromatography—mass spectromentry. *J. Liq. Chromatogr. Relat. Technol.* **2010**, *33*, 1067–1081.

(20) Kruve, A.; Kunnapas, A.; Herodes, K.; Leito, I. Matrix effects in pesticide multi-residue analysis by liquid chromatography—mass spectrometry. *J. Chromatogr., A* **2008**, *1187*, 58–66.

(21) Niessen, W. M.; Manini, P.; Andreoli, R. Matrix effects in quantitative pesticide analysis using liquid chromatography—mass spectrometry. *Mass Spectrom. Rev.* **2006**, *25*, 881–899.

(22) WHO. Environmental Health Criteria 121 — Aldicarb International Programme on Chemical Safety (IPCS) Monograph, 1991; http://www.inchem.org/documents/ehc/ehc/ehc121.htm.