Leaching of Pesticides in Tea Brew

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A cup of tea that cheers can also be an important route of human exposure to pesticide residues. It is important to evaluate the percent transfer of pesticide residue from dried (made) tea to tea infusion, as tea is subjected to an infusion process prior to human consumption. To investigate the pesticide translocation, 13 pesticides commonly used on tea were studied by subjection of fortified teas to infusion. Analytes of interest were quantified by gas–liquid chromatography with nitrogen–phosphorus and electron capture detectors. Interestingly, water solubility of pesticides did not necessarily indicate a shift of residues toward their preferential accumulation in infusion. The pesticides with larger partition coefficient (K_{ow}) values remained nonextractable in infusing water. Further, boiling for longer periods (extended brewing time) resulted in higher transfer of pesticides to tea brew.

Keywords: *Pesticide; leaching; tea; infusion*

INTRODUCTION

Tea is the most widely consumed beverage in the world, and India is the largest producer as well as consumer of tea. Tea represents a significant potential source of human exposure to pesticide residues by virtue of high application of pesticides to tea crop coupled with the average intake of six grams of dried (made) tea per day per individual (*1, 2*). Pesticides used on tea plants during cultivation, such as ethion, quinalphos, malathion, and dimethoate, are sufficient to formulate firm residue levels in tea, and a hot tea brew may act as a solvent for many of these chemicals. Therefore, to protect the health of consumers, periodic assessment of pesticide residues in tea is necessary, as a common cup of tea may easily hold a combination of various groups of pesticide residues (*3*).

In recent years, there is an increasing public concern over the presence of pesticide residues in food products because of the known toxicity of pesticides (*4–7*), and tea is not an exception (*8*). Under mounting pressure from importing countries and environmental health professionals, it has become important to reassess the impact of pesticide residues in tea as per the recommended maximum residual limits (MRLs). Many of the pesticides listed for tea do not yet have firm limits assigned because insufficient field trial data are available. Therefore, for the time being, importing countries are free to maintain or set their own tentative limits (U.S. EPA) or to adopt stipulations fixed by an international organization such as CODEX, which sets standards for the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) (*9*). As a result, the existing inconsistencies in standards, on occasion, have resulted in problems with global tea trade.

The work done so far on pesticide residue aspects of tea is quite scarce, however, some published papers described the loss of organophosphates, pyrethroids, and γ-HCH and DDT residues in black or green tea (*10, 11*). Other papers described the determination of organochlorine residues in black tea (*12*) and in linden and camomile (*13, 14*), but not in their infusions. However, Nagayama (*3*) observed 2 to 52% transfers of residues of organophosphates into brewed tea.

The present investigation aims to study the transfer of pesticides from made tea to infusion that may help in critically reviewing the relevance of existing MRLs on tea, considering the fact that made tea is not consumed as such, it is subjected to infusion. Therefore, pesticide transfer to infusion should be considered for setting realistic MRLs. Further, the present study has evaluated the difference in pesticide transfer to infusion between traditional and international methods of tea preparation. In the traditional method people, especially the rural population of India, enjoy the much-brewed tea (i.e., long brewing time of 5–10 min) after a day's hard work.

MATERIALS AND METHODS

Reagents and Apparatus. *Analytical Standards and Working Solutions.* The following pesticides commonly used in tea cultivation were selected for the study: Group I organophosphates (quinalphos, phosphamidon, methyl parathion, chlorpyrifos, malathion, monocrotophos, ethion, and dimethoate); Group II organochlorines (endosulfan and dicofol); Group III synthetic pyrethroids (deltamethrin, cypermethrin, and permethrin).

Analytical standards of these pesticides were obtained from Sigma Chemical Co. (St. Louis, MO; purity >98%). Standard solutions (1000 mg/L) were prepared in acetone, and the spiking solutions of 50 mg/L were diluted from the stock solution. All the solvents and chemicals used were of analytical grade from E. Merck.

Apparatus. A gas–liquid chromatograph, Hewlett-Packard 5890 equipped with a nitrogen–phosphorus detector (NPD) and an electron capture detector (ECD), was used. Group I pesticides were detected with NPD, HP-1 cross-linked methyl silicone gum column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and the operating conditions were injector temper-

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Table 1. Recoveries (percent) of Pesticides under Study from Tea Infusion in Different Solvents[a]

pesticide	*n*-hexane	benzene	chloroform	dichloro-methane
chlorpyrifos	100	- - -[b]	- - -	- - -
ethion	98.5−102	103	- - -	98
malathion	91−103	99−102	92	100
methyl parathion	91−95	90−95	87	69
dimethoate	- - -	41−48	96−100	40
monocrotophos	90−95	- - -	- - -	- - -
phosphamidon	> 15	- - -	35	89−95
quinalphos	98−100	- - -	- - -	- - -
endosulfan	96	- - -	50	91
dicofol	100	- - -	- - -	- - -
deltamethrin	90−100	- - -	- - -	98
cypermethrin	92−95	- - -	- - -	95
permethrin	90−100	- - -	- - -	95

[a] Fortification level 50 mg/L. [b] - - -, not checked.

ature 220 °C; detector temperature 200 °C; oven temperature 150 °C for initial 2 min and then ramped 10 °C/min to 300 °C final temperature and held for 5 min; and carrier gas nitrogen with a flow rate of 20 mL/min. Groups II and III pesticides were detected with ECD, HP-17 cross-linked 50% phenyl methyl silicone column (25 m × 0.2 mm i.d. × 0.25 μm film thickness) operated at injector temperature 250 °C; detector temperature 300 °C; oven temperature 200 °C for initial 5 min and then ramped 4.0 °C/min to 280 °C and held for further 10 min; carrier gas nitrogen with a flow rate of 2.0 mL/min.

Florisil Column. Glass columns (30 cm × 1.1 cm i.d.) with Teflon stopcocks were packed from the bottom with glass wool plugs and 2 cm of activated Florisil (60−100 mesh) between the layers of anhydrous sodium sulfate.

Tea Leaves and Infusion. Made tea samples were manufactured in the laboratory's mini manufacturing unit with a conventional orthodox tea manufacturing process. The untreated green tea leaves (two and a bud) were collected from the Institute of Himalayan Bioresource Technology Experimental Tea Farm, *Banuri*, and withered under a gentle air stream for 12−15 h. They were mechanically rolled in a Piezy roller, fermented, and dried at 100° C in an oven. Dried (made) tea (5-g portions) in replication (1 control + 5 replicates) was fortified at 10 mg/kg separately with each pesticide under study and kept for 20 h for complete absorption of pesticides.

The fortified made tea of individual pesticide was subjected to the infusion process: 5 g of made tea was immersed in 200 mL of boiling water, and after 2 min of brewing the water extract was filtered through a 420-μm stainless steel filter, cooled, and transferred to a separatory funnel. The pesticide was partitioned (extracted) into *n*-hexane (2 × 100 mL). The organic layer was separated, combined and concentrated to 5 mL, and transferred to a Florisil column that was prewashed with *n*-hexane. The column was eluted with *n*-hexane (2 × 25 mL) followed by a mixture of dichloromethane and *n*-hexane (15:85, v/v). The combined eluate was concentrated to near dryness under reduced pressure below 40 °C. The residue was reconstituted in 2 mL of acetone for final analysis. The same procedure was followed for the extraction of all the pesticides, except for dimethoate and phosphamiden, which were extracted into chloroform and dichloromethane, respectively. The efficiency of partitioning solvents was confirmed initially in a dummy experiment performed with positive controls (without tea) using different solvents, and the solvent with maximum acceptable recovery (>90%) was selected for further recovery experiments from tea infusions.

To observe the effect of brewing, in a parallel experiment fortified made tea of each pesticide in replication (5 × 5 g) was infused in 200 mL of boiling water and heated for 2, 5, and 10 min. The same procedure described above was followed for extraction and cleanup.

Detection and Quantification. Quantification was accomplished by using standard curves prepared by diluting the stock solution in acetone where good linearities were achieved between the range 0.1 to 1.0 ng with correlation coefficients

Table 2. Water Solubility and Calculated K_{ow} Values of Pesticides under Study

pesticides	solubility mg/L	K_{ow}
chlorpyrifos	2.0	16255.5
ethion[a]	insoluble	N.A.[b]
malathion	145.0	156.0
methyl parathion	55−60	447.4
dimethoate	25000	0.6
monocrotophos	10000	1.6
phosphamidon	completely miscible	−
quinalphos	22.0	1208
endosulfan	insoluble	N.A.
dicofol	0.8	43954.2
deltamethrin	0.002	34506.0
cypermethrin	1.0	34506.4
permethrin	0.2	34434.2

[a] Source The Pesticide Manual, 8th edition, published by British Crop Protection Council. [b] N. A., not applicable.

**Table 3. Percent Transfer of Pesticide Residues to the Tea Brew[a]**

pesticide	concentration in made tea μg /ml	concentration in tea infusion μg/mL	% transfer to infusion
chlorpyrifos	50	1.57 ± 0.02	3.14 ± 0.06
ethion	50	0.4 ± 0.04	0.8 ± 0.02
malathion	50	6.07 ± 0.4	12.14 ± 0.8
methyl parathion	50	4.98 ± 0.55	9.96 ± 1.0
dimethoate	50	12.92 ± 1.12	25.8 ± 2.23
monocrotophos	50	9.89 ± 1.08	19.78 ± 3.76
phosphamidon	50	16.65 ± 0.38	33.3 ± 0.76
quinalphos	50	4.02 ± 0.18	8.04 ± 0.36
endosulfan	50	0.9 ± 0.0	1.80 ± 0.06
dicofol	50	0.052 ± 0.0	0.10 ± 0.01
deltamethrin	50	N. D.[b]	−
cypermethrin	50	0.03 ± 0.0	0.06 ± 0.0
permethrin	50	N. D.	−

[a] Number of replicates: 5. [b] N. D., not detected.

in the range of 0.9990−0.9999. Overall limit of sensitivity or detectability was estimated to be 0.05 ng of pesticides, and was based on a signal-to-noise ratio >2:1. The column was conditioned by repeated injections (2−4) of a mixture of standard/sample extracts until GC residue peaks were reproducible.

RESULTS AND DISCUSSION

Recoveries for all the pesticides were determined at different concentrations for tea samples that had been prechecked to confirm the absence of any pesticide. Recoveries were measured by comparing peak areas of the recovery samples with external standards in acetone and a matrix-matched control prepared from unfortified tea extracts. Table 1 represents the recoveries of pesticides from infusion using different solvents. The hexane extract recovered most of the organochlorines, organophosphorus, and synthetic pyrethroids, except dimethoate which was best recovered in chloroform (96−100%) and phosphamidon which was best recovered in dichloromethane (89−95%). On the basis of the principle that like dissolves like, the extraction efficiencies of the solvents may be compared by their respective polarities when solvent/water pair is taken. Dimethoate and phosphamidon, being highly polar, are best extracted from water (tea brew) with relatively polar solvents (chloroform and dichloromethane), whereas less polar pesticides were best extracted into *n*-hexane. Moreover, the water immiscible nature of the solvent was also considered while choosing a solvent for quantitative extraction because their own solubility in water recover-

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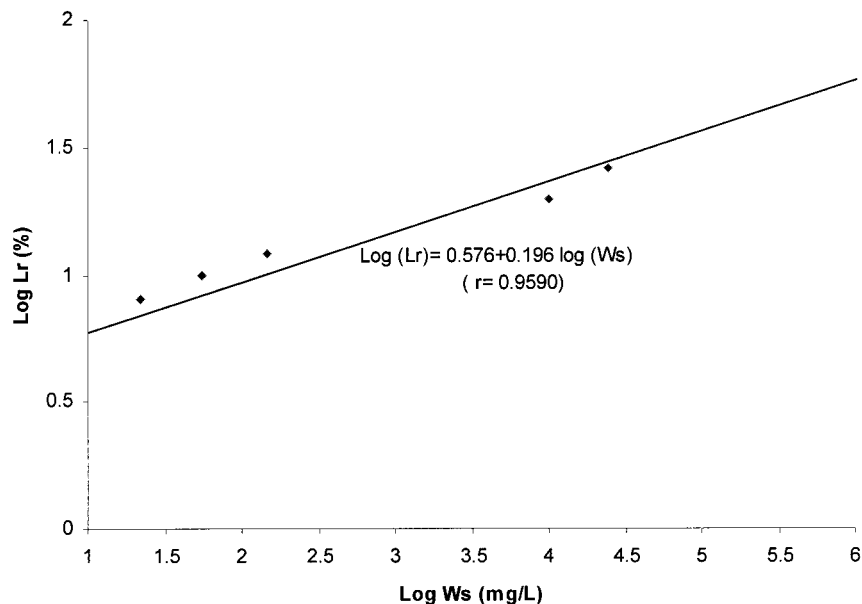
Figure 1. Relationship between water solubility and leaching ratio.
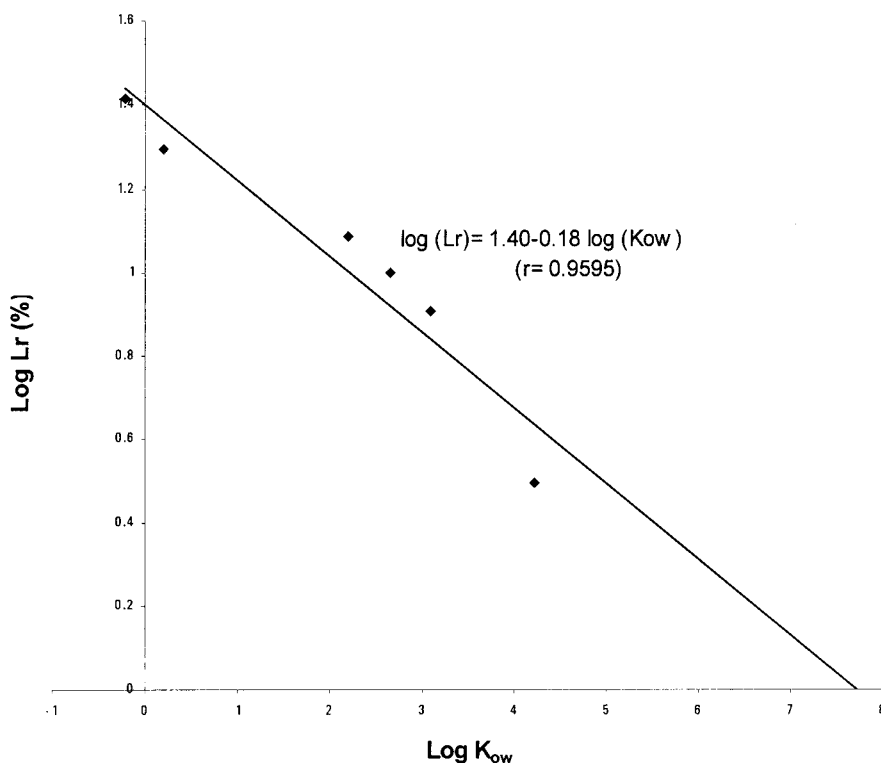
Figure 2. Relationship between leaching ratio and partition coefficient.

ies are generally suppressed, thus affecting the partitioning ability (Table 2).

The results of the transfer of pesticides from tea leaves (made tea) to brew are shown in Table 3. It was observed that phosphamidon translocation to the tea infusions was highest (33.3%), followed by dimethoate (25.8%) and monocrotophos (19.78%), whereas the transfer rates of malathion (12.14%), methyl parathion (9.96%), quinalphos (8.04%), and chlorpyrifos (3.14%) were comparatively less. Extractabilities of ethion, dicofol, endosulfan, deltamethrin, cypermethrin, and permethrin in infusion were almost negligible or below the detection limits (Table 3). To a great extent, the transfer of pesticides to the brew can be attributed to their water solubilities (Table 2).

The extraction rate of pesticide residues in water depends on their water solubility (*10, 15*) and the leaching ratio varies with the type of pesticide but not with the residue concentration (*15*). However, for the present study, the fortification concentration was kept constant to allow a similar rate of pesticide translocation. The transfer of pesticides under study primarily appeared to be dependent on their water solubilities. The water insoluble pesticides (organochlorines as well as synthetic pyrethroids), as expected, showed their negligible transfer to infusion, whereas polarity of most of the organophosphates resulted in their significant transfer to the infusions (Table 3). Although water solubility plays an important role, the results obtained were not necessarily dependent on this factor alone.

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Table 4. Percent Transfer of Pesticides to Tea Brew at Different Brewing Times[a]

	percent transfer of pesticides to tea brew		
pesticide	2 min	5 min	10 min
chlorpyrifos	4.0 ± 0.75	6.7 ± 1.2	10.9 ± 1.4
ethion	0.7 ± 0.14	0.9 ± 0.08	1.16 ± 0.02
malathion	10.2 ± 3.0	13.38 ± 1.0	13.8 ± 1.0
methyl parathion	10.9 ± 1.8	14.4 ± 1.2	18.26 ± 0.6
dimethoate	26.2 ± 1.2	30.12 ± 2.0	32.5 ± 0.8
monocrotophos	20.0 ± 2.5	26.12 ± 1.6	26.0 ± 0.82
phosphamidon	35.5 ± 4.2	38.0 ± 0.6	40.2 ± 0.2
quinalphos	10.0 ± 1.6	11.5 ± 0.2	14.8 ± 1.6
endosulfan	1.5 ± 0.32	0.5 ± 0.06	0.0
dicofol	0.1 ± 0.02	1.28 ± 0.02	1.8 ± 0.08
deltamethrin	0.0	0.24 ± 0.00	0.28 ± 0.0
cypermethrin	0.1 ± 0.22	0.4 ± 0.02	0.32 ± 0.06
permethrin	0.0	0.44 ± 0.00	0.48 ± 0.0

[a] Number of replicates: 5.

Nagayama (*15*) has explained a satisfactory relationship between water solubility and partition coefficient (K_{ow}) wherein pesticides with large K_{ow} values entered the tissue, strongly combining with it, and did not move with the circulating water, thus making it insoluble in the water extracts of made tea. The calculated K_{ow} values of the pesticides under study are presented in Table 2. For insoluble pesticides, the K_{ow} values were found to be highest when their solubility was equal to zero or negligible. As evident, phosphamidon with lowest K_{ow} (because of highest solubility), showed maximum transfer to the infusion. But pesticides of Groups II and III, which have comparatively larger K_{ow} values, remained in the tea leaves; this was also reported by Tsumura-Hasegawa et al. (*16*). The pesticides in tea leaves are partitioned between the leaves and the infusion depending on their water solubility (*10, 11−17*). The present data also substantiate a satisfactory relationship between the pesticide transfer to brewed tea (i.e., leaching ratio (Lr)) and water solubility (Ws) and partition coefficient (K_{ow}) as shown in Figures 1 and 2, respectively.

It was observed that the increase in brewing time resulted in an increased transfer of pesticides from made tea to brew for the cases studied (Table 4). It was further interesting to observe that those compounds which were undetectable (owing to their lower water solubility) at 2 min of brewing were well within the detection limits for the infusions at 5 and 10 min of brewing. In fact, with increased brewing time, concentration of a pesticide in water may increase or decrease depending upon its fugacity and volatility proportions (*18*). Pesticides of Groups II and III with low water solubility will have partial pressure approximately equal to their vapor pressure, and their concentration in water approximate to their water solubility as per Henry's constant (*19*). Hence, the low volatile compound will show maximum transfer to infusion, as observed for dimethoate and phosphamidon. Further, in the diluted solution of pesticide brew, the solubility is directly temperature-dependent; as a result, the water insoluble pesticides (such as the synthetic pyrethroids) showed their existence at longer duration of brewing. The chances of degradation of the pesticides of Groups II and III from codistillation are comparatively less because of their low vapor pressure; whereas the high vapor pressure of organophosphates (Group I) might have resulted in their degradation to some extent. Chen and Wan (*20*) have observed that the rate of loss of organophosphates is between 30 and 70% and is due to their high vapor pressure. Further, the chemicals with high molecular weight are generally less soluble in hot water within 2−3 min. Increasing the brewing time and continuous heating may aid in releasing these pesticides from the plant tissue. Thus, the traditional practice of over-boiling the tea leaves should be discouraged, as there may be the chance of more transfer of pesticides from made tea to brew.

CONCLUSION

It may be concluded that the mere presence of pesticide residues in made tea does not necessarily mean that the tea has become toxic and would pose a health hazard. The present study revealed that only a negligible or small percentage of pesticides in made tea is being transferred to the infusion. Further, the extent of pesticide leaching depends on its water solubility, partition coefficient, and the brewing time.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Chen, Q. Tea − The best drinking for health. *China Tea* **1985**, *3*, 28.

(2) Deng, Z.; Tao, B.; Li, X. Effect of tea on the living time of *Musca domesticas* and antistress of KM Rats. *J. Nanchang Univ.* **1997**, *2*, 69−72.

(3) Nagayama, T.; Maki, T.; Kan, K.; Lida, M.; Tamura, Y.; Nishima, T. Residues of organophosphorus pesticides in commercial tea and their leaching into tea. *Nippon Noyaku Gakkaishi* **1989**, *14*, 39−45.

(4) Handley, H. K.; Cairns, T.; Luke, M. A.; Masumoto, H. T. Pesticide residues findings by the Luke method in domestic and imported foods and animal feeds for fiscal years 1982−1986. *J. Assoc. Off. Anal. Chem.* **1988,** *71*, 875−92.

(5) Nagayama, T.; Kobayashi, M.; Shioda, H.; Tamura, Y. Pesticide residues in imported raw agricultural commodity. *J. Food Hyg. Soc. Jpn.* **1994,** *35*, 652−660.

(6) Nagayama, T.; Kobayashi, M.; Shioda, H.; Tamura, Y.; Pesticide residues in imported raw agricultural commodity. *J. Food Hyg. Soc. Jpn. (Shokuhin Eiseigaku Zasshi)* **1995**, *36*, 643−655.

(7) Neidert, E.; Saschenbreker, P. W. Occurrence of pesticide residues in selected agricultural food commodities available in Canada. *J. Assoc. Off. Anal. Chem.* **1996**, *79*, 549−566.

(8) Singh, R. P.; Agnihotri, N. P. Residues of dicofol, endosulfan, and malathion on tea *Camellia sinesis* L. *J. Entomol. Res.* **1984**, *8*, 14−16.

(9) Choudhuri, T. C. Pesticide residues in tea. In *Global Advances in Tea Science;* Jain, N. K., Ed.; Aravali Books Inernational (P) Ltd: New Delhi, India, 1999; pp 369−378.

(10) Wan, H.; Xia, H.; Chen, Z. Extraction of pesticide residue in tea by water during the infusion process. *Food Addit. Contam.* **1991**, *8*, 497−500.

(11) Zongmao, C.; Haibin, W. Factors affecting residues of pesticides in tea. *Pestic. Sci.* **1988**, *23*, 109−118.

(12) Peterson, J. H.; Jenson, K. G. Pesticide residues in black tea. *Z. Lebensm.-Unters. Forsch.* **1986**, *182*, 489−491.

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(13) Fernandez, N.; Sierra, M.; Garcia, J. J.; Diez, M. J.; Teran, M. T. Organochlorine pesticide residues in black tea, Camomile, and Linden. *Bull. Environ. Contam. Toxicol.* **1993**, *50*, 479−485.

(14) Carisano, A.; Rovida, C. SFE-facilitated detection of pesticide residues in Camomile. *LC−GC Int.* **1995**, *8*, 334−337.

(15) Nagayama, T. Behavior of residual organophosphorus pesticides in foodstuffs during leaching or cooking. *J. Agric. Food Chem.* **1996**, *44*, 2388−2393.

(16) Tsumura-Hasegawa, Y.; Tonogai, Y.; Nakamura, Y.; Ito, T. Residues of post-harvest application of pesticides in citrus fruits after storage and processing into lemon marmalade. *Shokuhin Eisegaku Zasshi* **1992**, *33*, 258−266.

(17) Zimmerli, B.; Blaser, O. Preliminary study of the transfer of pesticides from tea leaves to the infusion. *Mitt. Geb. Lebensmittelunters. Hyg.* **1982**, *73*, 174−185.

(18) Sunito, L. R.; Shiu, W. Y.; Mackay, D.; Seibes. J. N.; Glofelty, D. Critical review of Henry's law constants for pesticides. *Rev. Environ. Contam. Toxicol.* **1988**, *103*, 1−59.

(19) Kusk, K. O. General principles for mobility and abiotic degradation of chemical compounds. http://www.imt.dtu.dk/courses/63336/KAPS-00.htm. Dec., 1997.

(20) Chen, Z. M.; Wan, H. B. Factors affecting residues of pesticides in tea. *Pestic. Sci.* **1986**, *23*, 109−118.

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