Abstract

Background/Objectives: The residual pesticide problem even in Herbal Medicinal Materials (HDMs) has increasingly brought concerns to the patients (consumers). Methods/Statistical analysis: For the estimation of pesticides’ exposure to consumers via herbal drugs, it must be required that the transfer rates (TRs%) of the residual pesticides from raw to the final herbal drug, occurring via processing steps, be determined. The TRs% of 13 pesticides belonging to 6 groups were investigated from 7 HDMs by employing independent drying and/or decoction steps. Residues were quantitated in dried HDMs and decoctions using GC-ECD. Findings: The fate of the pesticide in the HDM (after drying and decoction) was mainly dependent on the nature of the pesticide, and not on the nature of the HDM. However, among the five HDMs, Daechu showed the most universal holding capability for the all tested pesticides even after drying process. The low cumulative average TR% (up to 0.4%), of drying and decoction, was obtained from mevinphos, chlorpyrifos, p,p’-DDE and diazinon, whereas chlorfenapyr (34.3%), bifenthrin (54.3%) and fenvalerate (56.7%) had high cumulative average TR%. Improvements/Application: This TR% s of residual pesticides shall be used for the establishment of sound and cost-effective HDMs management system in the future.

Keywords: Decoction, Drying, Fate, Herbal Drug Materials, Pesticides, Transfer rate

1. Introduction

Herbal medicine, a kind of Traditional Medicine (TM) is widely used and of rapidly growing health system and economic importance. In Korea, the total gross of Herbal Drug (HD) product was $3,000,000,000 in. TM accounts for around 37~41% of all health care delivered in China. Herbal drug materials (HDMs) have the equivocal characteristics of foods and drug. In ancient times, humans used plants as medicine and foods throughout the world. Especially the tradition of medicinal usage of the liquid (mainly water) extract of the dried plants has been continued in East Asia such as Korea, China and Japan.

Recently, the consumers (patients) of herbal medicine have been getting more concern about some intentionally applied hazardous components during cultivation and preservation of HDMs. The use of pesticide for the HD plants may provoke the frequent commitment of illegal acts due to the lack of registered pesticides for the HD plants, typical minor crops where the costs involved in generating data for registration. However the use of pesticides for HD plants may remain the lower residual pesticides on the HDMs than its original HD, after drying and decoction (typical HD processing steps in East Asia). The residues on raw edible commodities (foods and HDMs) are normally dissipated during storage, transport preparation, commercial processing, and cooking. Some pesticides are destroyed by preparation processes, such as drying, heating and boiling. During HD processing, in some cases, pesticide residues cannot be dissipated due to their high stability. As a result, the amounts of pesticide residues in HD products are not decreased than those in raw HDMs.

Therefore, for an accurate estimation of consumers’ exposure to pesticide via HD products, it is necessary that the transfer rates (TR%) of the residual pesticides from raw HDMs to the final HD products, occurring via HD processing steps, be determined. In the present study, the TR% s of 13 pesticides belonging to 6 groups were inves-
tigated from 7 HDMs by employing independent drying and/or decoction steps. These rates can be used to establish the pesticide Maximum Residue Limits (MRLs) of HDMs for the independent regulation under the food pesticide MRL system.

2. Experimental

2.1 Materials and Sample Preparation

Thirteen pesticide standards (belong to five chemical families) above the purity 95% were purchased from Chem Service, INC (West Chester, PA, USA), Dr. Ehrenstorfer (Augsburg, Germany) and Waco pure chemical Industries (Osaka, Japan) (Table 1). Each pesticide standard was dissolved in acetone as a stock solution (1000 mg L\(^{-1}\)) and preserved under the -4 °C within 3 months. For the experiment of drying, five raw HDMs were tested (Table 2). Raw HDMs were acquired from local producers in South Korea directly. Three dried HDMs were purchased from Gyoungdong market, Seoul, South Korea for the decoction experiment. They were identified by an herbal medicine expert. The Korean/Latin and academic names with parts used are presented in Table 2. Some HDMs has the separate Korean names depending on the status (raw or dried).

For drying test, 50 g of each Raw HDM (Chic, Saengang, Daechu, Gamcho and Gugija) was fortified with the thirteen pesticides (50-100 µg each) dissolved in acetone (10 mL) kept for 48 hours at room temperature (27 ± 2 °C) followed by drying at dry oven (50 °C) for 48 hours. The residual pesticide concentration was measured before and after drying. To get the water loss factor, each HDM was weighed before and after drying. The water loss factor was calculated as dividing the weight of dried HDM by the weight of raw HDM.

The official pesticides analysis method for herbal drug etc. No. 1 announced by Korea Food and Drug Administration (The official notification 2010-75, revised in October 29\(^{th}\), 2010) was applied for the analysis of residual pesticides in HDMs and its decoction. For the analysis of dried sample (5 g), 40 mL of distilled water was added and kept for 4-hours at room temperature. The moisturized sample was ground mechanically to homogeneous matter (by Omni mixer homogenizer, Model17105, Marietta, GA, USA) with acetone (90 mL) and filtered. The filtrate was partitioned with dichloromethane (70 mL) after addition of the mixture of saturated salted water (50 mL) and distilled water (100 mL). The separated upper water layer was saturated with sodium chloride and extracted with dichloromethane (70 mL) again. The separated down layers (organic solvents) were combined and concentrated to near dryness under the vacuum (by N-EVAP111 of Organomation Associates, Inc., Berlin, MA, USA) followed by reconstitution with \(n\)-hexane (4

Table 1. Chemical properties of the tested pesticides

| Active substance | Chemical family | Biological Classification | Partition Coefficient \(\log K_{ow}\) | Water solubility (mg/L) | Vapor Pressure mPa |
|------------------|----------------|--------------------------|--------------------------------|------------------------|-------------------|
| Iprodione        | Dicarboximide  | Fungicide                | 3                              | 13 at 20°C             | <0.133 at 20°C    |
| Vinclozolin      | Dicarboximide  | Fungicide                | 3                              | 3.4 at 20°C            | 0.016 at 20°C     |
| Dicofol          | Organochlorine | Acaricide                | 4.3                            | 0.8 at 25°C            | Negligible        |
| p,p’-DDE         | Organochlorine | Insecticide              | 6.96                           | 0.003 at 25°C          | 0.867 at 20°C     |
| p,p’-DDT         | Organochlorine | Insecticide              | 6.914                          | < 1 at 20°C            | 0.025 at 25°C     |
| Mevinphos        | Organophosphate| Insecticide              | 0.127                          | 6 × 10\(^{-5}\) at 20°C| 17 at 20°C        |
| Malathion        | Organophosphate| Insecticide              | 2.75                           | 130                    | 0.671 at 25°C     |
| Diazinon         | Organophosphate| Insecticide              | 3.3                            | 40 at 20°C             | 0.097 at 20°C     |
| Phenthoate       | Organophosphate| Insecticide              | 3.69                           | 11 at 24°C             | 5.33 at 40°C      |
| Chlorpyrifos     | Organophosphate| Insecticide              | 4.7                            | 2 at 25°C              | 2.5 at 25°C       |
| Fenvalerate      | Pyrethroid     | Insecticide              | 5.01                           | 0.002 at 25°C          | 1.467×10\(^{-5}\) at 25°C |
| Bifenthrin       | Pyrethroid     | Insecticide Acaricide    | 6                              | 0.1 at 25°C            | 2.41×10\(^{-5}\) at 25°C |
| Chlorfenapyr     | Pyrroles       | Insecticide Miticide     | 4.83                           | 0.00012 at 25°C        | 1.333×10\(^{-5}\) at 25°C |
It was transferred to a florisil cartridge column (6 mL, 1g) that was prewashed with n-hexane and acetone. The column was eluted with 5 mL of a mixture (50:48.5:1.5) of n-hexane, dichloromethane and acetone. The internal standard (4,4-dibromobiphenyl) was added. The eluate was concentrated to near dryness under reduced pressure below 40 °C. The residue was reconstituted in 2 mL of 20% acetone in n-hexane for the instrumental analysis.

For the decoction test, each 50 g of dried HDM powders (Gugija, Mansam and Hwangbaek) were fortified with the twelve pesticides (50 µg each) dissolved in acetone (10 mL) except p,p’-DDT then kept for 48 hours at room temperature (27 ± 2°C). Decoction was made in an Herbal brewing pot (DWP-3800T, Daewoong, South Korea) with 50 g of the (pesticides fortified) dried HDM and 500 mL of distilled water that was subjected to boil for 5-hours (which is a traditional decoction method in Korea). The decoction (under 50 mL due to evaporation of water during decoction) was centrifuged (7500 g, 30 min, 4°C). The supernatant (adjusted to 50 mL with distilled water) was subjected to the partition with acetone (90 mL), dichloromethane (70 mL x 2), saturated salted water (50 mL) and distilled water (40 mL). The following solid-phase extraction using the florisil cartridge column was same to the above.

### 2.2 Instrumental

Gas chromatograph-micro electron capture detector (GC-µECD) was used for the instrumental analysis. The instrumental conditions were same to former research. For validating the efficiencies of the method, the recovery tests were performed with dried Gugija after spiking the thirteen pesticides with the internal standard (at concentration 1 µg g⁻¹). The calculation method is based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula: LOQ = 10 × (SD/S). The standard deviation of the response is determined based on the standard deviation of y-intercepts of regression lines. The transfer rate (TR%) was calculated as the percentage of the final concentration of pesticide after drying or decoction. In case of TR% of drying experiment, water loss factor was multiplied to the final concentration of the pesticide.

### 3. Results

#### 3.1 The Optimization of Pesticide Analysis

The thirteen pesticides were well separated on baseline except chlorpyrifos and dicofol (Figure. 1). Therefore peak area results were cross-checked with the peak height results. The Limit Of Quantification (LOQ) of each pesticide spiked to Gugija was from 0.01 µg g⁻¹ (chlorpyrifos, etc.) to 0.10 µg g⁻¹ (mevinphos). The matrix matched recovery rate was from 72.6% (vinclozolin) to 86.7% (DDT). The relative standard deviation (RSD%) determined from triplicate analyses of each pesticide was under 10% except DDE (11.7%) and DDT (15.8%) (Table 3).

#### 3.2 Pesticide Dissipation during Drying Process

During the drying process, although the loss of water led to an increase in the theoretical amount of the pesticide
The TR% of dicarboxide pesticides (vinclozolin and iprodione) were comparatively low (average 6.0–6.5%). Organophosphate and organochlorine pesticides showed a large variation in the TR% (average, 0.3–19.0%). Interestingly, during the drying process, most of p,p'-DDE had dissipated, while comparatively large amount of p,p'-DDT was transferred (average 19.0%). The reason for this could be the lower vapor pressure of p,p'-DDT (0.025 mPa at 20 °C) than of p,p'-DDE (0.867 mPa at 20 °C). Low vapor pressure protects the pesticide from fugacity. While chlorpyrifos and p,p'-DDE showed the highest leaching degrees, pyrethroid pesticides (fenvalerate and bifenthrin) were transferred after drying with high survivability. The vapor pressure of chlorpyrifos is 2.5 mPa at 25 °C that ranks bifenthrin, fenvalerate, chlorfenapyr, etc. The residual amounts of phenthoate, and mevinphos (having a higher vapor pressure than that of chlorpyrifos) in the HDMs were unexpectedly larger than the amount of chlorpyrifos in the HDMs after the drying process (Table 4). However, the rate of evaporation (fugacity) is directly proportional to Henry's law constant ($H$). The $H$ of chlorpyrifos is much higher (several order of magnitude) than mevinphos, phenthoate and malathion. On the other hand, according to the JMPR (Joint Meeting of the FAO Panel of Pesticide Residues) report, when grapes containing chlorpyrifos residue at concentrations of 1.3 or 0.38 mg kg$^{-1}$ were sun dried, the processing factors for raisins were on an average 0.21 (TR%, 21) possibly due to the sticky matrix characteristics of raisins with high sugar contents. The TR% of HDMs' drying process for the pesticides such as fenvalerate (32.9%), mevinphos (7.4%) and bifenthrin (39.0%) with low RSD% (7–36%, that means there was no difference of TR% depending on the matrices) could be used as a processing factor for the establishment of pesticide maximum residue limit (MRL) of HDMs. The average TR% of the 13 pesticides in each HDM ranged from 7.8% (Gugija) to 23.5% (Chic) with RSD% from 66% (Daechu) to 134% (Gugija). During the drying process, raw HDM Chic and Daechu appeared as the matrices retaining the highest pesticide content among the HDMs. Especially Daechu retained all pesticides over the LOQ levels with RSD%, 66% that is the lowest level among the five HDMs. That means the survivability of all pesticides was highest in Daechu. Daechu is used in Korean and Chinese traditional medicine, where they are believed to alleviate stress. Daechu is the Jujube fruit that was domesticated.
Table 4. Transfer rates (%) of pesticides after drying for the herbal medicinal materials

| No | Pesticides   | Before drying (mg kg⁻¹) | After drying (mg kg⁻¹) | Transfer rate (%) |
|----|--------------|--------------------------|------------------------|-------------------|
|    |              | Chic | Saeng-gang | Daechu | Gamcho | Gugija | Chic | Saeng-gang | Daechu | Gamcho | Gugija | Aver- age | RSD (%) |
| 1  | Chlorpyrifos | 1.00± | 0.084 | 0.101 | 0.075 | 0.066 | 0.91± | 0.000 | 0.000 | 0.04± | 0.010 | 0.000 | 0.000 | 0.000 | 0.000 | 0.3± | 0.084 |
| 2  | DDE          | 0.92± | 0.094 | 0.090 | 0.070 | 0.051 | 0.78± | 0.000 | 0.000 | 0.07± | 0.021 | 0.000 | 0.000 | 0.000 | 0.000 | 0.0± | 0.000 |
| 3  | Iprodione    | 1.23± | 0.114 | 0.045 | 0.075 | 0.166 | 0.49± | 0.049 | 0.000 | 0.47± | 0.119 | 0.000 | 0.000 | 0.000 | 0.000 | 7.6± | 0.114 |
| 4  | Vinclozolin  | 1.02± | 0.113 | 0.087 | 0.101 | 0.097 | 0.99± | 0.021 | 0.06± | 0.141 | 0.06± | 0.024 | 0.07± | 0.041 | 5.6± | 0.113 |
| 5  | Mevinphos    | 1.23± | 0.086 | 0.170 | 0.136 | 0.111 | 1.15± | 0.032 | 0.42± | 0.083 | 0.32± | 0.093 | 0.27± | 0.083 | 9.8± | 0.086 |
| 6  | Dicofol      | 1.43± | 0.129 | 0.144 | 0.134 | 0.111 | 1.53± | 0.022 | 0.06± | 0.000 | 0.54± | 0.183 | 1.15± | 0.109 | 2.6± | 0.129 |
| 7  | Malathion    | 1.21± | 0.098 | 0.121 | 0.091 | 0.059 | 0.31± | 0.071 | 0.31± | 0.128 | 0.17± | 0.173 | 0.52± | 0.200 | 17.3± | 0.098 |
| 8  | Diazinon     | 0.97± | 0.120 | 0.138 | 0.082 | 0.097 | 0.83± | 0.019 | 0.86± | 0.090 | 0.04± | 0.019 | 0.35± | 0.088 | 59.2± | 0.120 |
| 9  | Phenthoate   | 1.08± | 0.102 | 0.118 | 0.088 | 0.111 | 0.91± | 0.014 | 0.27± | 0.132 | 0.27± | 0.063 | 0.11± | 0.028 | 22.0± | 0.102 |
| 10 | DDT          | 0.79± | 0.065 | 0.130 | 0.099 | 0.056 | 0.89± | 0.012 | 0.64± | 0.146 | 0.26± | 0.085 | 0.33± | 0.131 | 33.0± | 0.065 |
| 11 | Chlorfenapyr | 1.04± | 0.111 | 0.089 | 0.071 | 0.070 | 1.02± | 0.019 | 0.89± | 0.208 | 0.65± | 0.217 | 0.48± | 0.129 | 57.2± | 0.111 |
| 12 | Fenvalerate  | 1.50± | 0.112 | 0.084 | 0.055 | 0.172 | 1.07± | 0.164 | 1.30± | 0.224 | 2.48± | 0.476 | 1.85± | 0.207 | 28.9± | 0.112 |
| 13 | Bifenthrin   | 0.95± | 0.087 | 0.078 | 0.068 | 0.061 | 0.76± | 0.158 | 1.02± | 0.141 | 1.11± | 0.186 | 0.91± | 0.135 | 62.6± | 0.087 |
|    | Average      | 1.11± | 1.16± | 1.04± | 1.24± | 0.95± | 0.37± | 0.36± | 0.52± | 0.53± | 0.37± | 14.7± | 103

*Transfer rate (%) was calculated as (the pesticide concentration after drying × water loss factor × 100 ÷ the pesticide concentration before drying). The water loss factor for the five HDMs such as Chic, Saeng-gang, Daechu, Gamcho and Gugija was 0.668, 0.308, 0.358, 0.332 and 0.202, respectively.

Table 5. Transfer rates (%) of pesticides after decoction for the herbal medicinal materials

| No | Pesticides | Before decoction (mg kg⁻¹) | After decoction (mg kg⁻¹) | Transfer rate (%) |
|----|------------|-----------------------------|---------------------------|-------------------|
|    |            | Gugija | Mansam | Hwangback | Gugija | Mansam | Hwangback | Gugija | Mansam | Hwangback | Average |
| 1  | Chlorpyrifos | 0.43± | 0.052 | 0.60± | 0.072 | 0.33± | 0.039 | 0.05± | 0.002 | 0.08± | 0.003 | 0.04± | 0.003 | 11.3± | 13.3± | 11.9± | 12.2± | 1.0± | 0.05± |
| 2  | DDE         | 0.86± | 0.069 | 0.95± | 0.076 | 0.75± | 0.060 | 0.19± | 0.004 | 0.22± | 0.006 | 0.17± | 0.008 | 22.0± | 23.4± | 22.7± | 22.0± | 0.7± | 0.069 |
| 3  | Iprodione   | 0.81± | 0.058 | 1.00± | 0.070 | 0.91± | 0.063 | 0.31± | 0.010 | 0.44± | 0.025 | 0.37± | 0.012 | 37.8± | 44.1± | 40.8± | 40.7± | 3.4± | 0.058 |
| 4  | Vinclozolin | 0.47± | 0.052 | 0.22± | 0.024 | 0.14± | 0.015 | 0.06± | 0.004 | 0.03± | 0.005 | 0.02± | 0.005 | 13.1± | 12.8± | 12.6± | 12.8± | 0.3± | 0.052 |
| 5  | Mevinphos   | 1.32± | 0.092 | 1.60± | 0.112 | 1.12± | 0.078 | 0.00± | 0.000 | 0.00± | 0.000 | 0.00± | 0.000 | 0.0± | 0.000 | 0.0± | 0.000 | 0.0± | 0.000 |
| 6  | Dicofol     | 0.22± | 0.023 | 0.27± | 0.041 | 0.19± | 0.029 | 0.03± | 0.007 | 0.05± | 0.008 | 0.04± | 0.006 | 14.0± | 19.6± | 19.1± | 17.6± | 3.1± | 0.023 |

*Not tested*
in south Asia by 9000 BCE. Ziziphus jujuba, commonly called jujube (sometimes jujuba), red date, Chinese date, Korean date, or Indian date is a species of Ziziphus in the buckthorn family (Rhamnaceae), used primarily as a shade tree that also bears fruit. Daechu, Jujube fruit is often left to become wrinkled and spongy after drying, which increases their sweetness. The characteristically sticky matrix and the hard surface of Daechu may retain more pesticides than other HDMs during drying process.

3.3 Pesticide Dissipation during Decoction

The TR% of the 12 pesticides from different dried HDMs to their respective decoctions are shown in Table 5. The average TR% of each pesticide in 3 HDMs (in decoction) ranged from 0% (mevinphos) to 56.8% (fenvalerate). The average TR% of 12 pesticides in each HDM was quite similar to each other: 23% (Gugija and Hwangbaek) and 25% (Mansam). This implies that the matrix properties might not influence the pesticide's fate in the decoction. According to Tewary et al., the extraction efficiency of the pesticides in decoction is proportional to their respective solubility in water, that is, inversely proportional to their partition coefficient. However, pesticides with low water solubility (0.1 mg L⁻¹ or less), such as chlorfenapyr, fenvalerate, p,p'-DDE, and bifenthrin, showed comparatively higher average TR% (over 20%) during decoction (Table 5). The 5-hour decoction time (unlike 5 minutes in the study conducted by) might have caused higher dissipation of the pesticides (such as mevinphos, malathion and diazinon) via its rapid dissolution in the water phase (decoction) than the pesticides with low water solubility. Chlordiazepoxide, on the other hand, showed the average TR% (12%) almost identical to that of the peppermint tea infusion. Coulibaly and Smith reported chlordiazepoxide hydrolyzes by 53.2–80% even in unheated water. Two organophosphorus pesticides—diazinon and mevinphos—almost dissipated in decoction. Mevinphos readily dissolves in water and is easily broken down by water. Diazinon breakdown was reported to be rapid at higher water temperatures: ~2–4 times faster at 21 °C than at 10 °C.

3.4 Cumulative Pesticide Dissipation during Drying and Decoction

The cumulative average TR% of each pesticide was calculated as average TR% during HDM drying multiplied by average TR% of HDM decoction divided by 100. The low cumulative average TR% (up to 0.2%) was obtained from mevinphos (0%), chlorpyrifos (0%), p,p'-DDE (0.1%) and diazinon (0.2%), whereas chlorfenapyr (13.3%), fenvalerate (18.7%) and bifenthrin (19.6%) had high cumulative average TR%. The cumulative average TR% of others were 0.8% (vinclozolin), 1.4% (malathion), 1.5% (dichloro-diphenyl-trichloroethane (DDE), and bifenthrin etc.) of the pesticides were dissipated. The average TR% (during drying process) of the pesticides such as fenvalerate, mevinphos and bifenthrin were comparatively consistent for the tested HDMs (with RSD% 7–36%). However, other pesticides’ TR% during drying of HDMs varied largely depending on the nature of the HDMs’ matrices (RSD% 53–224%). Among the five HDMs, Daechu showed the most universal holding capability for the all tested pesticides even after drying process. During the decoction process, 35.7% (fenvalerate in Gugija) – 100% (mevinphos) of the pesticides were dissipated. The HDMs’ matrix properties might not influence the pesticide’s fate (the RSD% of pesticides ranged from 0-45.5%) in decoction. If the dissipation results of the pesticides during the drying and decoction processes were combined, 43.2% (bifenthrin)–100% of the pesticides were dissipated. In Korea, HDMs are mostly sold in dry form in the market, and 99.2% of HDMs are consumed as decoction. Therefore the TR% of residual pesticides must be carefully investigated because vigorous decoction conditions (boiling in hot water for a couple of hours) are unlike tea infusion, in which the plant materials are infused in hot water for just a couple of minutes. This TR% of residual pesticides shall be used for the establishment of sound and cost-effective HDMs management system in the future.

4. Conclusion

The present study showed that the average transfer of the pesticides (except mevinphos and diazinon) was lower during drying than decoction of HDMs. During the drying process, 37.4% (bifenthrin in Chic) – 100% (chlorpyrifos, DDE, iprodione etc.) of the pesticides were dissipated. The average TR% during drying process of the pesticides such as fenvalerate, mevinphos and bifenthrin were comparatively consistent for the tested HDMs (with RSD% 7–36%). However, other pesticides’ TR% during drying of HDMs varied largely depending on the nature of the HDMs’ matrices (RSD% 53–224%). Among the five HDMs, Daechu showed the most universal holding capability for all tested pesticides even after drying process. During the decoction process, 35.7% (fenvalerate in Gugija) – 100% (mevinphos) of the pesticides were dissipated. The HDMs’ matrix properties might not influence the pesticide’s fate (the RSD% of pesticides ranged from 0-45.5%) in decoction. If the dissipation results of the pesticides during the drying and decoction processes were combined, 43.2% (bifenthrin)–100% of the pesticides were dissipated. In Korea, HDMs are mostly sold in dry form in the market, and 99.2% of HDMs are consumed as decoction. Therefore the TR% of residual pesticides must be carefully investigated because vigorous decoction conditions (boiling in hot water for a couple of hours) are unlike tea infusion, in which the plant materials are infused in hot water for just a couple of minutes. This TR% of residual pesticides shall be used for the establishment of sound and cost-effective HDMs management system in the future.

5. Acknowledgment

This research was partly supported by Korea Food & Drug Administration. The author thanks S.H. Song for supporting sample preparation for the analysis.
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