Loss of Organochlorine Pesticide Residues during the Infusion Processes of Linden (*Tilia cordata* Mill.)

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Losses of residues of α-hexachlorocyclohexane (α-HCH), *β*-HCH, *γ*-HCH, hexachlorobenzene (HCB), heptachlor and its epoxide, *p*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDT, *p*,*p*′-DDT, α-endosulfan, *β*-endosulfan, endosulfan sulfate, aldrin, dieldrin, and endrin, when linden is subjected to three infusion processes, were studied. Compounds were quantified by gas−liquid chromatography with electron capture detection. Higher losses are found for heptachlor epoxide (HE) and *o*,*p*′-DDT, in process 1 and for α-HCH, HCB, HE, and aldrin in procedures 2 and 3. Lower losses are obtained for *β*-HCH in process 2. The best procedure for infusion preparation was procedure 3.

Keywords: *Pesticides; Tilia cordata Mill.; infusion processes*

INTRODUCTION

Linden (*Tilia cordata* Mill.) is a medicinal plant that is widely used by the Portuguese population. Inflorescences and leaves are prepared by infusion. Calming and sedative effects, by oral ingestion, and softening and antipruriginous actions, by external use, are cited. Different types of pesticide residues may be present in medicinal plants. They are distinct between individual countries, but organochlorine pesticides still exist as pollutants in countries like Portugal, despite their prohibition 8 years ago (Decree 660/88).

As tea is submitted to an infusion process prior to human consumption, it is therefore important to evaluate the percentage loss of pesticide residues during this process. Some published papers described losses of organophosphorus and pyrethroids and only *γ*-HCH and DDT residues in black or green tea (Wan et al., 1991; Zongmao and Haibin, 1988) and also in *Livesticum officinale* K and *Tenacetum balsamita* L. (Miellet, 1982). Other papers describe the determination of organochlorine pesticide residues in black tea (Peterson and Jensen, 1986) and in linden and in camomile (Fernández et al., 1993; Carisano and Rovida, 1995), but not in their infusions. In the present work, we intend to evaluate the loss of residues of insecticides like HCH isomers (α, *β*, *γ*), DDT group (*p*′,*p*′- and *o*,*p*′-DDT, *p*,*p*′-DDE, and *p*,*p*′-DDD), aldrin, dieldrin and endrin, heptachlor and its epoxide (HE), endosulfan (α, *β*, and sulfate), and a fungicide, HCB, using three different processes of linden infusion preparation.

EXPERIMENTAL PROCEDURES

Reagents. Sodium sulfate, anhydrous granulated for residue analysis (Merck), and *n*-hexane and dichloromethane (Carlo Erba, Italy) were of pesticide residue grade; water was purified via Milli Q (Millipore, Bedford, MA); pesticide standards were obtained from Dr. Ehrenstorfer (West Germany); elution solvents, *n*-hexane and dichloromethane−*n*-hexane (15 + 85), were prepared daily.

Materials and Apparatus. A gas−liquid chromatograph, Carlo Erba Mega HRGC 5300 equipped with a ^{63}Ni electron capture detector, was used. Two fused silica capillary columns, 30 m × 0.25 mm × 0.25 μm, with chemically bonded phases DB-5 and DB-17 (J&W Scientific), were used. The first one was used for quantification and the second as a confirmation column. In both columns, 1 μL of sample was injected in the splitless mode, and the splitter was opened after 60 s. Chromatographic conditions were at temperatures of 280 °C for the detector, 220 °C for the injector, and 150 °C held for 1 min and programmed at 10 °C/min to 210 °C held for 1 min and programmed at 3 °C/min to 230 °C, held for 5 min and finally programmed at 3 °C/min to 250 °C, held for 3 min to the first column and for 10 min to the second column. Gases used were as follows: carrier gas helium N60 carrier at 2 mL/mim, split valve 100 mL/min, purge valve 2 mL/min, makeup gas, nitrogen at 120 kPa. For quantification, a Spectra-Physics 4270 integrator was used, which compared peak areas in samples and standard solutions. A mechanical shaker for the separatory funnel, Agitelec (J. Toulemond, Paris), was used. The water bath was set to ∼35 °C. Other equipment was also used: nitrogen U for extract concentration, vacuum system for SPE and vacuum pump B-160 Vacobox Büchi (Switzerland), centrifuge Pyrex test tubes with PTFE-lined screw caps from Schott (West Germany), pyriform flasks (50 mL), rotary vacuum evaporator (Heidolph VV 2001), and filter paper Whatman no. 4 (Maidstone, England) washed with acetone.

All Florisil glass cartridges (6 mL) (J. T. Baker, Phillipsburg, NJ) were from the same lot. Linden samples composed by inflorescences and leaves were collected from open-field trees.

Sample Preparation. The process of drying was conducted in an oven at 40 °C. The dry sample was cut into very small pieces with scissors, ground to a powder in a mortar with a pestle, and then homogenized.

Infusion Processes. Infusions were prepared with tap water, as in the usual process of infusion preparation, and the same kettle (13.4 × 13.8 cm i.d.) was used in all studies. Three different infusion processes were carried out. Each procedure was done in triplicate, using 2 g of linden. Process 1: In the first, one mL of a pesticide standard mixture was added to linden in the same concentration used for recovery study. After being left to stand 15 min, linden was placed in the kettle containing 200 mL of tap water. After the water boiled, it was allowed to stand for 3 min. It was then cooled in a recipient containing ice−water. Process 2: The second procedure was similar, but instead of placing linden in the water, 200 mL of tap water was first boiled. After the kettle was turned off, spiked linden was added to the water and allowed to stand for 3 min. It was cooled in a manner similar to that described in the previous procedure. Process 3: In the third process, the linden was placed in a 600 mL cup, and after addition of standard mixture and standing for 15 min, 200 mL of boiling tap water was added to it. It was allowed to stand for 3 min and cooled in ice−water.

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Table 1. Recovery Mean (CV) (%) (n = 5) of 16 Organochlorine Pesticides from Linden Infusion

compd	concn in linden (μg kg^{-1})	mean (CV) (%)
α-HCH	25	109 (9)
HCB	250	96 (5)
β-HCH	25	96 (13)
γ-HCH	75	91 (13)
heptachlor	125	82 (18)
aldrin	250	88 (9)
heptachlor epoxide	125	49 (17)
α-endosulfan	50	88 (4)
p,p'-DDE	625	76 (2)
dieldrin	125	78 (6)
endrin	250	81 (22)
β-endosulfan	100	34 (15)
p,p'-DDD	625	83 (8)
o,p'-DDT	625	77 (6)
endosulfan sulfate	100	39 (24)
p,p'-DDT	625	81 (6)

To verify the effect of heat on a standard solution of organochlorine pesticide residues, 1 mL of that solution was added to 200 mL of tap water and heated in a similar procedure (100 °C, 3 min).

Recovery Study. A linden infusion was prepared by adding the linden (15 g) to boiling water (1500 mL), providing the same proportion used for different infusion processes (200 mL of water/2 g of linden), for 3 min. It was cooled as in the previous description. It was filtered through paper. To evaluate the recovery of pesticide residues in this matrix, 1 mL of a standard solution (corresponding to 25 μg/kg of α-HCH and to 625 μg/kg of DDT, isomers, and analogs) (Table 1) was added to 200 mL of linden tea over five replications. Two samples of 200 mL of linden tea were used like sample blanks. These samples were submitted to the whole procedure (extraction method and SPE cleanup). The results of the obtained recoveries and coefficients of variation are presented in Table 1.

Extraction Method. After filtration of the linden infusion through paper and washing of the cake with water, the water extract was placed into a 500 mL separatory funnel containing 20 mL of *n*-hexane. Then, the separatory funnel was shaken for 2 min and the *n*-hexane phase passed through anhydrous sodium sulfate to 50 mL pyriform flasks. The aqueous phase was reextracted with 20 mL of *n*-hexane. The *n*-hexane phases were concentrated in a vacuum evaporator (~35 °C) to 3 mL.

SPE Cleanup. A similar SPE cleanup for *n*-hexane extracts of linden samples (Lino and Silveira, 1997) was used for the extraction of the linden infusion. It was made by adding 1 cm of sodium sulfate to a Florisil cartridge and washing the column with 10 mL of *n*-hexane without letting the column dry. The concentrated *n*-hexane extract was transferred to the column. It was allowed to flow by gravity. Two different eluents, E_1, 2 × 5 mL of *n*-hexane, E_2, 2 × 5 mL of dichloromethane−*n*-hexane (15 + 85), were used. The eluates were collected in a graduated centrifuge tube. The eluates were concentrated to 1 mL for quantification by GC−ECD.

RESULTS AND DISCUSSION

Figure 1 presents chromatograms obtained in the DB-5 column of a standard solution of organochlorine pesticides, of an organic extract of water containing organochlorine pesticides after boiling at 100 °C for 3 min, and of an organic extract of linden infusion made by procedure 3.

The extraction was made with *n*-hexane, and the cleanup of the supernatants was conducted using Florisil cartridges for *n*-hexane extracts because of the abundance in pigments that can raise problems for HRGC-ECD detection (Lino and Silveira, 1997). Sample extracts containing the organochlorine pesticide residues were analyzed on a GC system with DB-5 and DB-17 columns. The first column separated the 16 compounds, and in the second column p,p'-DDD and o,p'-DDT coeluted.

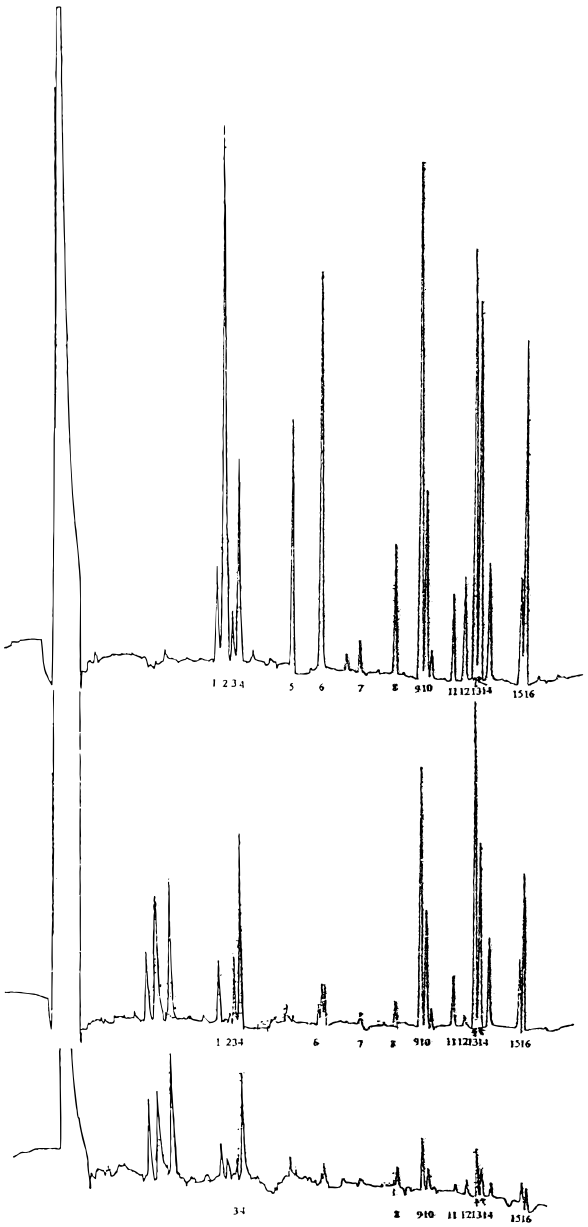

Figure 1. Typical chromatograms, on a DB-5 column, of organochlorine pesticides: (a) standard solution; (b) organic extract of water containing organochlorine pesticides after boiling at 100 °C for 3 min; (c) organic extract of linden infusion made by procedure 3.

The results of the recovery studies (Table 1) observed for linden tea are good for most compounds except for HE, β-endosulfan, and endosulfan sulfate, for which recoveries are 49%, 34%, and 39%, respectively. Low recovery levels were also found in linden matrix for β-endosulfan (15%), endosulfan sulfate (65%), and HE, which was not detected (Lino and Silveira, 1997).

The results of the infusion procedures 1−3 are listed in Tables 2−4, respectively, and in Figures 2 and 3.

In infusion procedure 1 (Table 2) we can observe that between the HCH isomers, the β presented lower losses, 48.90%, and α-HCH the higher percentage loss, 73.42%. Heptachlor epoxide and o,p'-DDT remained on the linden. They were not present in the infusion. The percentage loss in this procedure is up to 90% for HCB,

Table 2. Loss of OC Pesticide Residues Using Infusion Procedure 1

compd	concn in linden (μg kg^{-1})	concn in linden infusion (μg kg^{-1})	% loss
α-HCH	25	6.64	73.42
HCB	250	6.95	97.23
β-HCH	25	11.98	48.90
γ-HCH	75	26.31	64.91
heptachlor	125	12.42	90.04
aldrin	250	11.95	95.22
heptachlor epoxide	125	nd	100.00
α-endosulfan	50	10.85	78.30
p,p′-DDE	625	31.72	94.04
dieldrin	125	45.02	63.98
endrin	250	32.47	87.01
β-endosulfan	100	13.60	86.40
p,p′-DDD	625	77.33	87.63
o,p′-DDT	625	nd	100.00
endosulfan sulfate	100	49.45	50.56
p,p′-DDT	625	63.22	96.78

Table 3. Loss of OC Pesticide Residues Using Infusion Procedure 2

compd	concn in linden (μg kg^{-1})	concn in linden infusion (μg kg^{-1})	% loss
α-HCH	25	nd	100.00
HCB	250	nd	100.00
β-HCH	25	23.66	5.37
γ-HCH	75	17.38	76.83
heptachlor	125	nd	100.00
aldrin	250	nd	100.00
heptachlor epoxide	125	nd	100.00
α-endosulfan	50	31.36	37.28
p,p′-DDE	625	40.08	93.77
dieldrin	125	19.05	84.76
endrin	250	15.65	93.74
β-endosulfan	100	7.13	92.87
p,p′-DDD	625	33.52	92.79
o,p′-DDT	625	nd	100.00
endosulfan sulfate	100	16.06	83.94
p,p′-DDT	625	22.75	96.36

Table 4. Loss of OC Pesticide Residues Using Infusion Procedure 3

compd	concn in linden (μg kg^{-1})	concn in linden infusion (μg kg^{-1})	% loss
α-HCH	25	nd	100.00
HCB	250	nd	100.00
β-HCH	25	19.72	21.11
γ-HCH	75	53.00	29.34
heptachlor	125	nd	100.00
aldrin	250	nd	100.00
heptachlor epoxide	125	nd	100.00
α-endosulfan	50	7.37	85.26
p,p′-DDE	625	31.59	94.95
dieldrin	125	7.65	93.85
endrin	250	15.13	92.60
β-endosulfan	100	7.63	92.41
p,p′-DDD	625	53.31	91.47
o,p′-DDT	625	33.94	94.57
endosulfan sulfate	100	10.82	89.18
p,p′-DDT	625	56.60	90.95

heptachlor, aldrin, *p,p′*-DDE, and *p,p′*-DDT. The percentage loss for *p,p′*-DDD was higher than 80%. The same happened with endrin and β-endosulfan. Endosulfan sulfate presented the lowest percentage loss, 50.56%.

The most elevated losses for the majority of the 16 compounds in the study were observed in procedures 2 and 3 (Tables 3 and 4), with the exception of the β-HCH, which presented the lower percentage of losses, 21.11%, in procedure 3, and 5.37%, in procedure 2, despite its lower water solubility in comparison with other HCH isomers (Table 6). That fact may be due to its lower fugacity and volatibility proportion (Suntio *et al*., 1988), which lead to the volatilization of α and γ isomers when the infusion occurs. Also, γ-HCH is an exception because it presented less percentage losses in procedure 3, 29.34%. Hexachlorobenzene was lost completely in procedures 2 and 3 and almost completely in procedure 1, 97.23%, which is according to their water solubility in spite of its lower fugacity. The loss of heptachlor epoxide was complete in all three of the infusion procedures. With respect to DDT isomers, losses of *o,p′*-DDT in procedures 1 and 2 were complete and in procedure 3 were higher than 90%. *p,p′*-DDT losses were similar in the three studied procedures, higher than 90%. *p,p′*-DDD presented percentage losses that were lower with relation to DDE and *p,p′*-DDT in the three studied procedures. This phenomena is in relation to its water solubilities (Table 6).

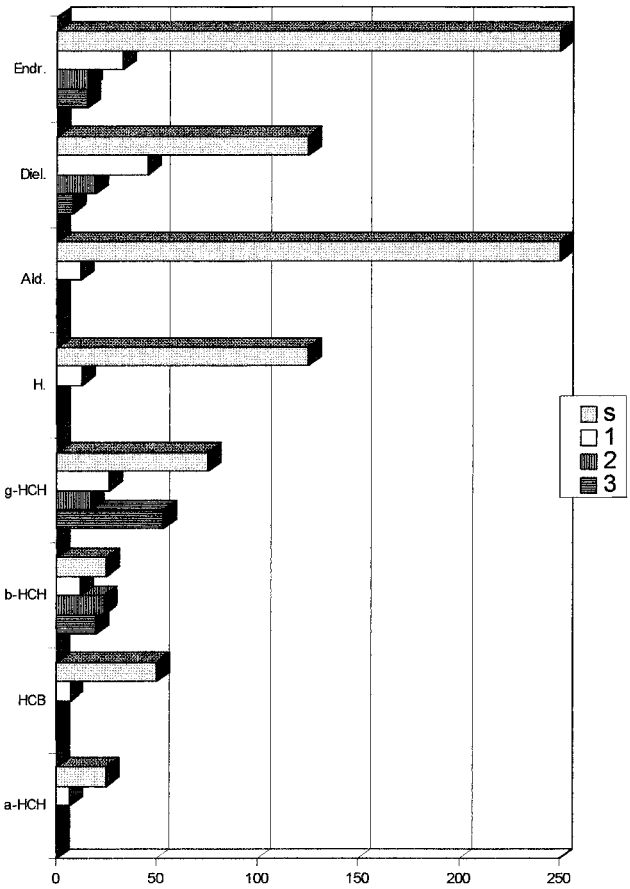

Figure 2. Graphic representation of organochlorine pesticide concentration in linden infusion (μg/kg) prepared by three different procedures: s, standard solution (top bar of each grouping); 1, procedure 1 (second bar of each grouping); 2, procedure 2 (third bar of each grouping); 3, procedure 3 (bottom bar of each grouping).

In procedure 3, only β- and γ-HCH presented losses higher than 20% but lower than 30%. Total losses occurred for α-HCH, HCB, heptachlor, aldrin, and HE. For the remaining compounds, the losses are higher than 85%. More elevated losses occurred for the vast majority of the compounds in procedure 3, followed closely by procedure 2.

With procedure 2, lower losses were verified for β-HCH, with 5.37%, followed by α-endosulfan, 37.28%, γ-HCH, 76.83%, and endosulfan sulfate and dieldrin with 83.94% and 84.76%, respectively. For the remain-

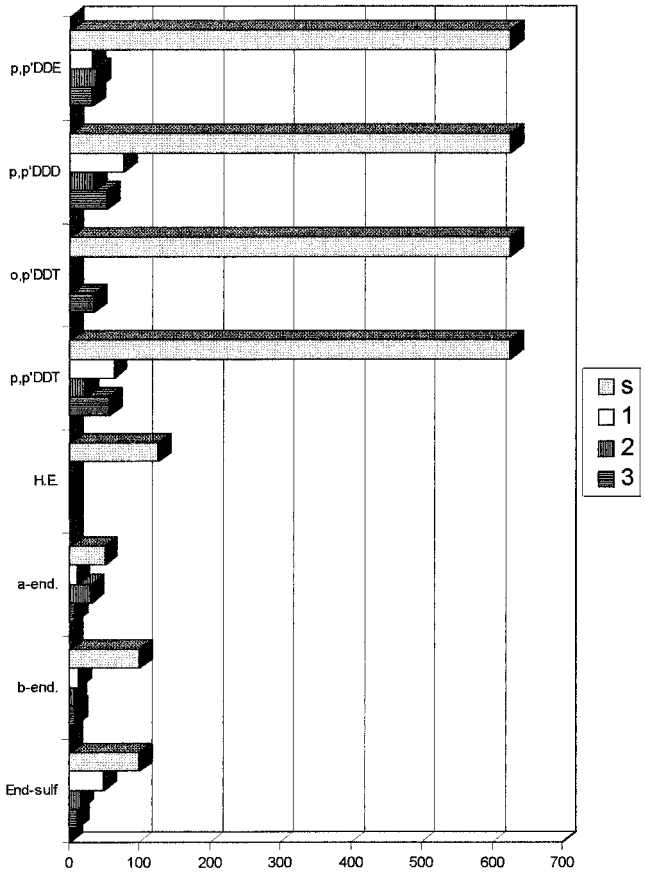

Figure 3. Graphic representation of organochlorine pesticide concentration in linden infusion (μg/kg) prepared by three different procedures: s, standard solution (top bar of each grouping); 1, procedure 1 (second bar of each grouping); 2, procedure 2 (third bar of each grouping); 3, procedure 3 (bottom bar of each grouping).

Table 5. Effect of Boiling (100 °C, 3 Min) on Standard Solutions of Organochlorine Pesticide Residues

compd	concn in linden (μg kg^{-1})	concn in linden infusion (μg kg^{-1})	% loss
α-HCH	25	23.55	5.80
HCB	250	2.95	98.82
β-HCH	25	14.00	44.00
γ-HCH	75	40.75	45.67
heptachlor	125	nd	100.00
aldrin	250	46.33	81.47
heptachlor epoxide	125	37.83	69.73
α-endosulfan	50	10.50	79.00
p,p′-DDE	625	207.67	66.77
dieldrin	125	45.25	63.80
endrin	250	131.17	47.53
β-endosulfan	100	9.03	90.97
p,p′-DDD	625	369.67	40.85
o,p′-DDT	625	230.17	63.17
endosulfan sulfate	100	67.00	33.00
p,p′-DDT	625	287.83	53.95

ing compounds, losses were up to 92.79%, and six of the compounds were completely lost.

According to the obtained results, procedure 1 seems to us the least recommended method for infusion preparation of linden because it presents the lowest percentage loss of most organochlorine pesticide residues in the study (with the exception of β-HCH in both procedures 2 and 3) and procedure 3 the most advised method due to the loss of compounds with the exception of γ-HCH, whose losses are higher in procedures 2 and 1.

Table 6. Water Solubility and Fugacity Ratio of Organochlorine Pesticides Involved in This Study (Suntio et al., 1988)

compd	water solubility (g/m^3 at 20 °C)	fugacity ratio (*F* at 20 °C)
α-HCH	1.0	0.041
HCB	0.04	0.0075
β-HCH	0.1	0.0012
γ-HCH	6.5	0.12
heptachlor	0.1	0.17
aldrin	0.02	0.14
heptachlor epoxide[c]		
α-endosulfan[a]	0.15	0.22
p,p′-DDE	0.04	0.2
dieldrin	0.17	0.026
endrin	0.23	0.013
β-endosulfan[a]		
p,p′-DDD	0.05	0.12
o,p′-DDT[b]		
endosulfan sulfate[a]		
p,p′-DDT[b]	0.003	0.13

[a] Refers only to endosulfan. [b] Refers only to DDT. [c] Not referred.

The effect of boiling (100 °C, 3 min) on standard solutions of organochlorine pesticide residues, observed in Table 5, shows us some elevated losses for heptachlor, 100%, HCB, 98.82%, β-endosulfan, 90.97%, aldrin, 81.47%, and α-endosulfan, 79%. Other compounds, like heptachlor epoxide, *p,p′*-DDE, dieldrin, and *o,p′*-DDT, presented losses higher than 60% but lower than 70%. With the exception of α-HCH, which presented losses of 5.8%, the remaining compounds revealed losses comprising between 33% and 53.95%.

Some investigators found an extraction rate of pesticide residues in water dependent on the water solubility. Their results indicate, for compounds like pyrethroids whose water solubility is lower than 1 mg/kg, that the extraction rate in black tea is 1−4% (Wan et al., 1991). That factor conditions the percent of loss of pesticide residues much more than the effect of chemical degradation, which is not important. Also Zongmao and Haiben (1988) verify that the amount of extracted pesticide of green and black tea depends on the water solubility, and a very high concentration of some pesticide in tea may present a very low concentration in the tea infusion because of that parameter and of degradation caused by the high temperature. In their study, they also found a very low percentage of extracted DDT (1%) and γ-HCH (6.5%). Compounds like DDT, isomers and analogs, aldrin, and HCB have the lower water solubility (Table 6). Also, the fugacity ratio of HCB is very low, 0.0075 F (Table 6).

In this work, besides water solubility (Wan et al., 1991; Zongmao and Haiben, 1988; Nagayama, 1996) and fugacity ratio influence, the procedure of infusion preparation is determinant in the pesticide losses not only by the different contact forms but also because chemical degradation takes place when pesticides are submitted to the effect of boiling (Table 5), which is confirmed by Zongmao and Haiben (1988).

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