Fast Microextraction by Demixture and Determination of Organochlorine Pesticides in Water

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A microextraction procedure by demixture using iso-propanol as organic phase and a mixture of $(NH_4)_2SO_4$ and $NaH_2PO_4.H_2O$ as salting agent is applied to the determination of 16 organochlorine pesticides in water. Under these conditions 120–200 µL of iso-propanol are separated. The concentration factor of the organic compounds from the aqueous phase ranges from 100 to 400. The analytical features and the results obtained in rain water and snow samples from the Pyrenees mountains in Spain (a wild protected area) are discussed. α-HCH and γ-HCH were found in almost all samples with concentrations ranging from 2 to 9 ng.L^{-1} and from 2 to 50 ng.L^{-1}, respectively.

INTRODUCTION

The high sensitivity necessary for the determination of organochlorine pesticides (OCPs) in environmental water samples is usually reached with a concentration step which involves the use of solid phase cartridges of Sep-pak type, solid membranes or liquid-liquid extraction and further evaporation of the final organic extract[1-4]. Recent trends in this area are the use of microcolumns coupled on-line to a sophisticated analytical system, the use of Empore-discs which can be used *in-situ* in the environmental sampling[5] and the use of the solid phase microextraction (SPME)[6]. However, these procedures have several problems. Liquid-liquid extraction involves a great consumption of organic solvents, which are expensive and have safety problems. On the other hand, the concentration step is often discriminatory for some pesticides and it concentrates the impurities of the solvents as well.

Further to these problems, another important fact is that both the liquid-liquid extraction and the concentration step on a solid phase commonly implies the use of large volumes of water. However, sometimes the water volume available (e.g. pore waters) is very limited and in such cases the necessary sensitivity values could not be reached.

An alternative procedure is the use of microextraction, in which the ratio between the organic and aqueous phase is very high. Several microextraction procedures have been proposed, one of them by EPA[7], but most of the times the results are only semiquantitative. The main problem affecting these methods is the distribution factor

between both organic and aqueous phases. Nevertheless, if the organic phase could be generated *in situ* in the aqueous phase through a demixture in a high ionic concentration, the mentioned problems could be overcome.

In a previous work such microextraction by demixture has been successfully applied for the determination of HCHs and chlorobenzenes in water[8]. The system consists of generating an organic phase (iso-propanol) *in situ* in an aqueous phase through a demixture in a high ionic concentration. The high ratio between the volume of the organic extract (120–200 μL) and the volume of the water sample (50 mL) allows the obtention of very high concentration factors in only one extraction step and, consequently, the determination of concentrations up to 2.5 $ng.L^{-1}$ in 50 mL of volume of water without requiring additional concentration steps.

This procedure is extended now to the determination of OCPs in rain and snow water samples, collected in the National Park of Ordesa and Monte Perdido (Northeast of Spain, Pyrenees mountains). This park has a total surface of 15,600 Ha, with a high between 3,335 and 750 m above sea level. The sharp mountains and valleys produce a great range of different climates, with wet precipitations between 800 and 2,000 mm, depending on the zone. To reflect this diversity, two sampling sites have been selected: Góriz (2,200 mm) and Tella (1,300 m above sea level).

EXPERIMENTAL

Apparatus and conditions

A Varian Star 3400 Cx Gas Chromatograph equipped with both split-splitless and SPI (on-column and temperature programmable) injectors, a Varian 8200 Cx autosampler and ECD ^{63}Ni as detector. The chromatographic data were obtained and processed in a Star Chromatography Workstation.

Two different capillary columns were used: SPB-5 (Supelco, Bellefonte, P.A., USA) 60 m × 0.25 mm i.d. × 0.25 μm film thickness and MFE-1701 (Análisis Vínicos, Tomelloso, Spain) 60 m × 0.25 mm i.d. × 0.25 μm film thickness. A 2 m × 0.32 mm i.d. pre-column (J & W. Scientific, Fisons) was used in both cases.

Chromatographic conditions

The following conditions were used: Splitless injection: injector temp. 210°C, splitless time 1 min. SPI injection: initial injector temp: 50°C, rate at $150°C.min^{-1}$ up to 250°C held for the rest of the analysis.

In both cases, the rest of conditions was as follows: Temperature program: 50°C held for 2 min, then raised to 185°C at $20°C.min^{-1}$, held for 10 min, raised to 280°C at $3°C.min^{-1}$. Final time = 6 min. Detector temp = 300°C; carrier gas: H_2 at 1.45 $mL.min^{-1}$, make-up gas: N_2 at 30 $mL.min^{-1}$.

Reagents and solutions

The following reagents were used: Iso-propanol of residue analysis quality (Merck, Darmstadt, Germany), anhydrous ammonium sulphate, monosodium 1-hydrate

phosphate and anhydrous sodium sulphate of analytical reagent grade from Panreac (Spain).

Water Milli-Q, with a resistivity value higher than 18 MΩ.cm., obtained from a Milli-Q purification system (Millipore).

The following standards were used: heptachlor, heptachlor-epoxide, α-endosulfan, endosulfan-sulphate and hexachlorobenzene were from Riedel de Häen (Cromlab, Spain). α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachlorocyclohexane (γ-HCH, lindane), dieldrin, endrin, aldrin, β-endosulfan, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT and chlorpiriphos were from Dr. Ehrensdorfer (Sugelabor, Spain). Endrin aldehyde was from Accu Standard. (Imatra, Spain)

Individual standard solutions in iso-propanol were obtained from standard solutions of 500 μg.g^{-1} in n-hexane by dilution with iso-propanol.

A standard iso-propanol solution containing all the compounds was prepared. Suitable dilutions of this standard solution were used when necessary.

Aqueous samples with known pesticide concentration were obtained by spiking Milli-Q water with a standard iso-propanol solution containing aprox. 500 μg.g^{-1} of each compound. The content of iso-propanol in the aqueous phase is in all cases negligible (e.g. 50 mL of an aqueous solution of 100 ng.L^{-1} contain only 10 nL of iso-propanol, without influence on the ternary equilibrium). All the standard solutions were stored at +4°C and gravimetrically controlled.

Procedure

The water sample (50 mL) was shaken in a separatory funnel with 37.71 g of $(NH_4)_2SO_4$, 6.85 g of $NaH_2PO_4.H_2O$, 10 μL of a tetrachloronaphtalene (TCN) and PCB-209 iso-propanol standard solution of 6.0 and 6.8 μg.g^{-1} respectively, and 1.2 mL of iso-propanol until the total dissolution of the salt (approx. 8 min). After holding for the separation of phases, about 120 μl of the organic phase was removed, with a Pasteur pipette, transferred to a microvial and injected into the gas chromatograph.

Aqueous solutions containing 5, 10, 20, 50, 100, 200 and 1000 ng.L^{-1} of each OCP as well as TCN and PCB-209, both used as internal standards, were used for calibration following the microextraction procedure.

Blank samples were prepared by extracting Milli-Q water sample in the same conditions as the unknown samples.

Recovery studies were carried out transferring the total organic demixed phase to a vial, washing the wall of the separatory funnel with iso-propanol and making up to a fixed weight (gravimetric control). These organic solutions were analyzed and the results were interpolated in a calibration plot previously obtained in iso-propanol. This procedure was applied to the mentioned OCPs as well as to TCN and PCB-209.

Collection of samples

All the samples were collected in 1 or 2.5 L brown glass bottles. The bottles were stored in a dark and cold place (+4°C) up to a maximum of 5 days before the analysis. Samples in both sampling sites were collected with a conventional stainless steel rain-meter adapted with a 30 cm diameter glass funnel. In both cases, the bottles were closed when it was not raining, to avoid the dry deposition of particulate matter. The bottles were immediately closed after the rain event.

RESULTS AND DISCUSSION

Microextraction by demixture

As it was previously demonstrated[8], the ternary system of water/ iso-propanol/ ammonium sulphate has an optimum point of demixture at a ratio of water to iso-propanol of 50:1.2 (v/v):37.71 g $(NH_4)_2SO_4$. Under these conditions, a small volume of about 120 μL of iso-propanol is obtained providing enrichment factors in the range 100–400, as will be discussed below.

Analytical performance

Table 1 shows the analytical features with regression coefficients, linear range, detection limits and quantification limits when the microextraction procedure is applied to all the compounds under study. The regression coefficients correspond to the calibration plots obtained by a microextraction of aqueous solutions within the linear range. As can be seen, all compounds are linear in the range of 5 ng.L^{-1} to 1 μg.L^{-1} with the only exception of aldrin, pp'-DDD and pp'-DDT in which the quantification limits are 20 ng.L^{-1} for the two former and 30 ng.L^{-1} for the latter, respectively. Lower and higher values were not studied for linearity. Detection limits (D. L.) are expressed as the concentration values in aqueous phase equivalent to three times the background signal of the chromatogram. The quantification limit was considered as the equivalent concentration to 10 times the background signal. In both cases, the background signal was measured after injecting into the capillary column the extract obtained when an ultrapure (Milli-Q) water sample was extracted under the same conditions described before.

The reproducibility of the procedure was studied at three different concentration levels, 5 ng.L^{-1}, 20 ng.L^{-1} and 100 ng.L^{-1}. Five independent analysis were carried out

Table 1   Regression coefficients (r), linear range, detection and quantification limits and RSD values for the determination of these compounds in water by microextraction by demixture. Concentration of each compound for RSD values: 100 ng.L^{-1}.

Compound	R. coef. (height)	RSD, % (n = 4)	D. Lim. ng.L^{-1}	Q. Lim. ng.L^{-1}	Linear range (μg.L^{-1})
α-HCH	0.9987	3.6	1	3	0.010-1
β-HCH	0.9962	6.0	2.5	8	0.010-1
γ-HCH	0.9986	6.1	1	3	0.010-1
δ-HCH	0.9960	6.2	2.5	8	0.010-1
Aldrin	0.9973	10.1	4	12	0.020-1
Heptachlor epoxide	0.9962	2.7	3	9	0.010-1
α-Endosulfan	0.9972	3.1	3	9	0.010-1
pp'-DDE	0.9994	3.6	3	9	0.010-1
Dieldrin	0.9972	6.3	3	9	0.010-1
Endrin	0.9970	4.5	3	9	0.010-1
β-Endosulfan	0.9932	3.1	3	9	0.010-1
pp'-DDD	0.9940	5.1	5	15	0.020-1
Endosulfan sulphate	0.9941	5.0	2.5	8	0.010-1
Endrin aldehyde	0.9966	4.9	2.5	8	0.010-1
pp'-DDT	0.9943	6.5	7.5	20	0.020-1
Heptachlor	0.9987	3.9	3	9	0.010-1

Table 2 RSD values obtained by the microextraction by demixture at three concentration levels (5 ng.L^{-1}, 20 ng.L^{-1} and 100 ng.L^{-1}). The results are the average of five independent replicates at each concentration level.

Compound	RSD values (%) (n = 5)		
	5 ng.L^{-1}	20 ng.L^{-1}	100 ng.L^{-1}
α-HCH	32.4	12.3	4.5
β-HCH	16.9	18.1	6.6
γ-HCH	37.4	15.6	5.0
δ-HCH	49.7	16.6	2.6
Aldrin	—	7.1	4.0
Heptachlor epoxide	30.6	16.4	1.4
α-Endosulfan	21.5	14.7	3.0
pp'-DDE	30.1	14.5	5.4
Dieldrin	40.7	16.6	5.6
Endrin	17.8	17.3	3.6
β-Endosulfan	19.8	25.5	4.7
pp'-DDD	—	14.9	9.5
Endosulfan sulphate	30.4	16.9	3.1
Endrin aldehyde	34.6	11.4	5.1
pp'-DDT	—	—	9.0
Heptachlor	—	18.0	3.5

in each case. Table 2 shows the relative standard deviations obtained. As can be seen, the results are quite good, considering the lower concentration values involved.
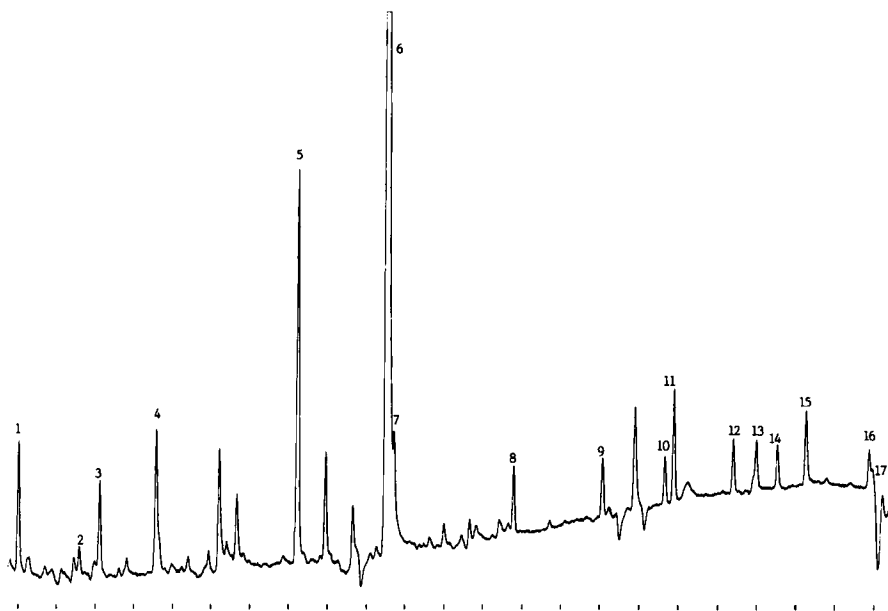
Figure 1 shows the chromatogram of the microextraction of a water sample containing about 20 ng.L^{-1} of the compounds under study. As it can be seen, no interferences were found, even though the concentration of OCPs in water was very low.

With respect to the behaviour of the ECD detector, previous studies[8] mentioned that some of the electron capture detectors available in the market could be damaged by the injection of the iso-propanol phase obtained by microextraction by demixture from a strongly salted sample. In our experience, more than 300 sequential injections of this iso-propanol demixtured sample were made in the ECD from Varian without any apparent problem.

Recovery studies and concentration factors

One of the main characteristics of this microextraction procedure is the high concentration factors which can be reached. As can be seen in Table 3, in all cases these factors are in the range of 100 to 400 with higher values for HCHs and lower for aldrin and dieldrin. It is important to point out that this factor is reached in only one step and without further evaporation to concentrate the final extract, which is very common in other analytical procedures. The absolute concentration factors vary with the little variations of the recovered organic phase, however these variations are compensated by the use of an internal standard at the beginning of the analytical process.

Table 3 shows the recovery values for several OCPs obtained by this procedure as well as the corresponding concentration factors in each case. As can be seen, recoveries

Figure 1 Chromatogram of a standard water sample obtained by GC-ECD after applying the microextraction by demixture procedure. Concentration of each compound in water sample: 20 ng.L^{-1}. *Peak identification*: 1: *α-HCH*, 2: *β-HCH*, 3: *γ-HCH*, 4: *δ-HCH*, 5: *Heptachlor*, 6: *Tetrachloronaphtalene (TCN)*, 7: *Aldrin*, 8: *Heptachlor epoxide*, 9: *α-endosulfan*, 10: *pp'-DDE*, 11: *Dieldrin*, 12: *Endrin*, 13: *β-endosulfan*, 14: *pp'-DDD*, 15: *Endrin aldehyde*, 16: *Endosulfan sulphate*, 17: *pp'-DDT*.

Table 3 Recoveries and concentration factors obtained in the microextraction by demixture procedure from 50 mL of water sample and 120 μL–200 μL of iso-propanol demixtured.

Compound	Recovery (%) ± S.D. (n = 3)	Concentration factor (1)
α-HCH	91.6 ± 5.9	229–382
β-HCH	96.4 ± 5.3	240–400
γ-HCH	94.3 ± 4.9	236–393
δ-HCH	97.2 ± 6.3	243–405
Heptachlor	45.1 ± 2.5	113–188
Aldrin	35.3 ± 2.1	88–147
Heptachlor epoxide	60.1 ± 4.3	150–250
α-Endosulfan	63.1 ± 3.5	158–263
pp'-DDE	55.9 ± 2.7	140–233
Dieldrin	38.6 ± 2.5	97–161
Endrin	69.5 ± 4.9	174–290
β-Endosulfan	57.7 ± 6.2	144–240
pp'-DDD	52.5 ± 2.3	131–219
Endosulfan sulphate	71.6 ± 2.8	179–298
Endrin aldehyde	80.8 ± 5.3	202–337
pp'-DDT	40.4 ± 1.2	101–168
Tetrachloronaphthalene (TCN)	97.4 ± 5.3	244–406
PCB-209	96.7 ± 1.6	242–402
4,4-Dibromobiphenyl	100.0 ± 1.3	250–415

(1) Range of the concentration factors, which depends on the variations of the volume of the final extract obtained in the interval of 120–200 μL.

ranging from 91 to 103% were obtained for HCHs, tetrachloronaphtalene and PCB 209, which can be considered as quantitative, whereas lower values were obtained for some of the OCPs under study such as aldrin or dieldrin. Nevertheless, the consequence of these lower recovery values is that the concentration factors are lower too. The reproducibility of this microextraction process, with RSD around 6% even for the mentioned OCPs, renders the method quantitative. The recovery data are not necessary to quantify the results obtained when the calibration plot is also obtained by microextraction by demixture of the standard aqueous solutions.

Determination of OCPs in rain water and snow

In order to test the described procedure for the analysis of natural environmental samples, a series of rain water and snow samples collected from a remote site in the Pyrenees were analyzed following the described microextraction procedure. Table 4 shows the results obtained in 13 different samplings carried out along 1995.

The only organochlorine pesticides appearing in all samples were α-HCH and γ-HCH at concentration values ranging from 2 to 9 ng.L^{-1} and from 2 to 50 ng.L^{-1} respectively. These concentrations are very similar to those found by other authors in the western Mediterranean[9] or other sites[10]. The concentrations of the other compounds were near or under the detection limit, and they could not be quantified. β-HCH, δ-HCH, heptachlor epoxide and α-endosulfan were detected in two samples and β-endosulfan, endosulfan-sulphate, pp'-DDE and pp'-DDT in only one sample but they could not be quantified.

Some relationships can be found out between the behaviour of these compounds and the seasonal and meteorological conditions in which the samples were monitored. The ratio of α-HCH/γ-HCH is an useful diagnostic indicator of both the recent usage versus old usage of HCH isomers and the distance from the input sources over which the HCH isomers may have been transported. When this ratio is about 3:1 it is indicative of environmental background levels[11]. In our samples it can be distinguished between the

Table 4 Results obtained for both the water and snow samples and meteorological conditions during sampling.

Sample	Date	Sampling site	α-HCH (ng.L^{-1})	γ-HCH (ng.L^{-1})	Ratio α-HCH/ γ-HCH	Wind speed,m.s^{-1} (Main direction)	Mean temp. (°C)	Rainfall (mm)
Snow	09-02-95	Goriz	8.7 ± 2.2	7.4 ± 2.1	1.18	5.0 (N)	−2.2	2.3
Snow	13-02-95	Goriz	6.2 ± 1.8	6.6 ± 2.0	0.94	3.2 (E)	−0.5	14.3
Snow	18-02-95	Goriz	2.1 ± 1.0	2.0 ± 1.0	1.05	23.1 (N)	−6.2	13.3
Snow	23-12-95	Goriz	4.6 ± 1.3	3.9 ± 1.6	1.18	8.5 (E)	0.2	100.0
Snow	24-12-95	Goriz	4.3 ± 1.3	2.7 ± 1.2	1.59	0.2 (W)	5.3	530.1
Rain	10-08-95	Goriz	2.8 ± 1.0	7.3 ± 2.1	0.38	2.7 (S)	10.4	15.6
Rain	20-08-95	Goriz	< Q.L.	< Q.L.	—	2.2 (S)	9.3	12.0
Rain	22-08-95	Goriz	3.0 ± 1.1	50.0 ± 3.5	0.06	3.9 (S)	15.2	14.3
Rain	22-08-95	Tella	2.7 ± 1.0	23.5 ± 3.7	0.11	5.4 (NE)	12.8	13.8
Rain	23-08-95	Tella	< Q.L.	42.5 ± 4.0	—	2.2 (SW)	14.5	1.6
Rain	04-10-95	Goriz	3.3 ± 1.1	7.4 ± 2.1	0.45	0.1 (S)	5.3	41.6
Rain	28-10-95	Goriz	5.1 ± 1.4	8.6 ± 2.3	0.59	5.6 (changeable)	3.4	18.4
Rain	22-12-95	Goriz	2.5 ± 0.9	4.7 ± 1.8	0.53	5.8 (N)	7.6	16.2

Q.L. : Quantification limit.

snow and the rain samples. In the snow samples, this ratio ranged from 0.9 to 1.6 and in the rain samples the ratio is lower and it ranged from 0.06 to 0.4. It can be observed that in the α-HCH concentrations there are not great differences between the snow and the rain samples, whereas the γ-HCH concentrations are higher in the rain samples. As snow samples were collected in cold weather while rain samples corresponded to warmer weather, these differences could be attributed to a seasonal influence. Two factors could contribute to these high concentrations of γ-HCH. Firstly, local seasonal agricultural activities with the usage of lindane that takes place mainly in spring and early summer and secondly, the higher environmental temperature of the Spring and Summer samples which would increase the volatilization of the γ-HCH from the treated surfaces. These results are in agreement with those found in other studies[11,12]. These works show that γ-HCH concentrations in the atmosphere are much higher during the warmer months and they are strongly dependent on temperature through the vapour pressure of the compound, whereas for α-HCH no temperature-concentration dependence was observed. Figure 2 shows the seasonal distribution of the α-HCH and γ-HCH concentrations and the correlations between air temperature and concentrations for both compounds.

On the other hand, it could not be observed an inverse correlation between rainfall volume and concentration in rainfall as has been mentioned by other studies, in which a great washout ratio at the beginning of the rainfall was observed[11-14]. Sample from 09-02-95 (snow) and Sample from 23-08-95 (rain) have the lowest rainfall with about 2 mm, but their OCPs concentrations are approximately the same as those obtained in the other samples having from 10 mm to 16 mm rainfall. This is probably due to the low rainfall volumes in both cases.
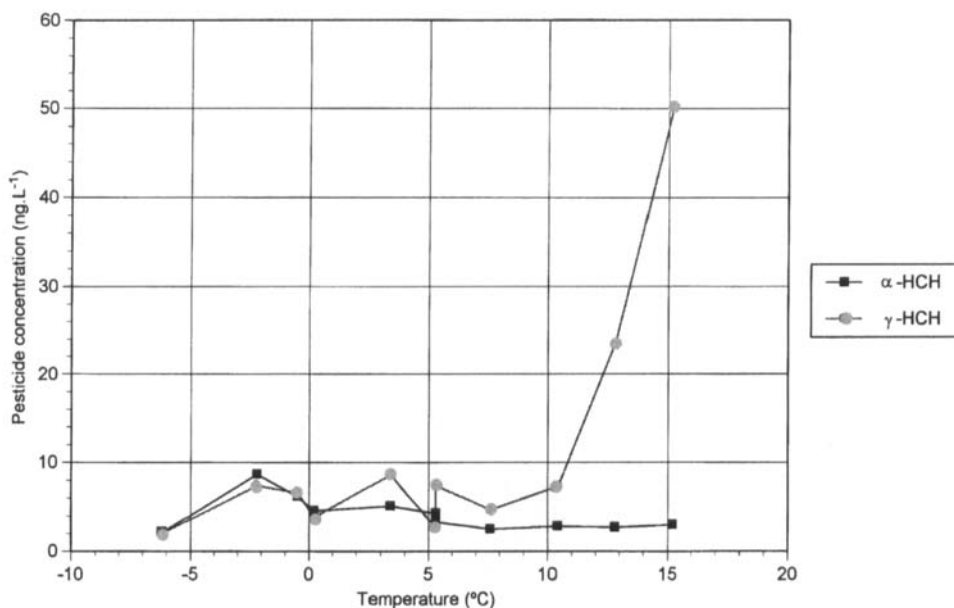
Figure 2 Air temperature during the sampling versus concentration of α-HCH and γ-HCH found in both rain water and snow samples.

CONCLUSIONS

The microextraction by demixture can be applied to the determination in water of the sixteen organochlorine pesticides studied (HCHs, DDTs, drins and others) with a good analytical performance. This analytical method is especially useful if the sample volume is scarce. The concentration factors as well as the recoveries obtained for most of the compounds under study are good enough to propose this analytical procedure for the analysis of OCPs at very low concentration levels of order of $ng.L^{-1}$.

The analysis of eight snow and rain samples from the National Park called Ordesa and Monte Perdido only showed α-HCH and γ-HCH in all the samples, with concentrations up to 9 $ng.L^{-1}$ for α-HCH and 50 $ng.L^{-1}$ for γ-HCH. Traces of β-HCH, δ-HCH, heptachlor epoxide and α-endosulfan were found in two samples and traces of β-endosulfan, pp'-DDE and pp'-DDT were found in only one sample. These results confirm that the atmosphere is one of the suppliers of organochlorine compounds to wild protected areas such as the National Park under study.

It could be observed that the concentration of γ-HCH in the rainfall is both strongly seasonal and temperature dependent, whereas the concentration of α-HCH shows none or little temperature- and seasonal-dependence.

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