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RESEARCH PAPER

Influence of pesticide physicochemical properties on the association between plasma and hair concentration

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Abstract Although the relationship between chemical intake and resulting concentration in hair remains incompletely elucidated, the transfer from blood to hair bulb living cells is generally considered the main route of incorporation. The present work investigated the correlation between blood and hair concentration of 23 pesticides/metabolites from different chemical classes in rats submitted to chronic controlled exposure. *Long-Evans* rats were administered pesticides by gavage three times per week over a 90-day period. After hair sample decontamination, pulverization, and extraction, compounds were analyzed by gas chromatography tandem mass spectrometry (GC-MS/MS). Blood was collected at sacrifice, immediately turned into plasma, and analyzed after extraction for the same compounds by GC-MS/MS. The data obtained for all the investigated compounds demonstrated significant association between plasma and hair concentrations (*P* value of 2.97E−45 and $R_{Pearson}$ of 0.875), with the exception of three outliers. For all the target compounds, water solubility, lipophilicity, molecular weight, and charge were therefore investigated in order to understand the role of these parameters in outliers' specific behavior. Although a possible change in the charge through the transfer from blood to hair might be suspected for two outliers, on the whole the physicochemical parameters investigated here did not seem to influence incorporation of chemicals into hair. Our results support that the concentration of chemicals in hair mainly depends on the respective concentration in plasma and suggest that for most compounds, the transfer from blood to hair would not represent a limiting step in the incorporation.

Keywords Pesticides · Hair · Plasma · Physicochemical properties

Introduction

According to a 2013 EFSA report, more than 600 scientific publications published after 2006 have established associations between pesticide exposure and diverse health outcomes with highlights on 23 major disease categories such as different types of cancer, diabetes, reproductive disorders, and neurological disease [1]. Among the different approaches aiming at assessing exposure, biomonitoring, consisting of the direct determination of pollutants (parent and/or metabolites) in biological matrices, presents the advantage of integrating all the possible sources and routes of exposure. In addition to the classical matrices such as urine and blood that were used to date for pesticide biomonitoring, increased interest have also been observed for hair analysis over the last 10 years [2]. In order to validate the use of hair analysis as a reliable matrix for the quantitative assessment of exposure, several studies have been conducted in the field of forensic toxicology on the relationship between dose and hair concentration: for instance, Ferko observed accumulation of cocaine and benzoylecgonine in rat hairs after several intraperitoneal administrations [3]; Scheidweiler demonstrated a dose-related concentration in hair following 10 weeks controlled cocaine

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and codeine administration on 10 volunteers [4]; Appenzeller showed a significant correlation between ethyl glucuronide in hair and the amount of alcohol intake with 15 subjects included in a withdrawal program [5]. In environmental toxicology, some studies have been conducted to compare concentration in hair and other tissues including blood [6]: Nakao et al. found significant correlations between hair and serum concentration for hexachloro dibenzo-p-dioxin (HxCDD), pentachloro dibenzofuran (PeCDF), pentachloro biphenyl (PeCB), and hexachloro biphenyl (HxCB) investigated in six incineration workers [7]. Altshul et al. investigated the relationship between blood and hair concentrations for some polychlorinated biphenyls (PCBs) and organochlorines from nine volunteers (four women, five men) and showed a good correlation only for persistent PCBs ($r = 0.6$) and for p,p′-DDE ($r = 0.8$) [8]. The relationship between intake and hair concentration, and between blood and hair concentration however seem to vary depending on compounds and the incorporation mechanisms of chemicals into hair remain incompletely understood. Despite a first insight from Nakahara on that aspect in the early 1990s, priority has afterwards been given to forensic or clinical applications of hair analysis to the detriment of mechanistic studies [9–13]. Since transfer from blood to living hair bulb cells is considered the main route of incorporation of chemicals into hair, the possible influence of compound physicochemical properties on the transport across cellular membrane has been questioned [14]. The study of the influence of physicochemical properties of compounds in their incorporation in hair requires controlled level of exposure and is therefore often limited to animal models, which offers the possibility to compare intake with chemical concentration in blood and in hair.

The present work investigated the correlation between blood and hair concentration of 23 pesticides and pesticide metabolites, including the most common chemical classes such as organochlorines, organophosphates, pyrethroids, and other miscellaneous pesticides. γ-Hexachlorocyclohexane (γ-HCH), β-hexachlorocyclohexane (β-HCH), β-endosulfan, p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT), p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), p,p′-dichlorodiphenyldichloroethane (p,p′-DDD), dieldrin, and pentachlorophenol (PCP) were representative of organochlorines. Diethylthiophosphate (DETP), diethylphosphate (DEP), and 3,5,6-trichloro-2-pyridinol (TCPY) were organophosphates. Pyrethroids were represented by cyhalothrin, permethrin, cypermethrin, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane carboxylic acid ($ClCF_3CA$), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cl_2CA), and 3-phenoxybenzoic acid (3-PBA). Two phenylpyrazoles, one oxadiazin, one carboxamide, one carbamate, and one anilin were also investigated; they were successively fipronil, its metabolite fipronil sulfone, oxadiazon, difluefenican, carbofuran phenol, and trifluralin. Moreover, the physicochemical parameters of the target compounds such as water solubility, lipophilicity (assessed by the octanol/water partition coefficient—Kow), and charge (extrapolated from the pKa) were investigated regarding their influence on the incorporation of chemicals from blood into hair.

Materials and methods

Chemicals

ULC/MS grade acetonitrile, analytical grade ethyl acetate, analytical grade methanol, and *n*-hexane were supplied from Biosolve (Dieuze, France). Ultrapure water was produced by an AFS-8 system from Millipore (Brussels, Belgium).

Analytical grade cyclohexane, polydimethylsiloxane-divinylbenzene (PDMS-DVB) fibers (65 μm film thickness), dibutyl phosphate (DBP), and the derivative agent 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) were purchased from Sigma-Aldrich (Diegem, Belgium). Hydrochloric acid (HCl 32 %) and salts like potassium carbonate (K_2CO_3), sodium dihydrogen phosphate monohydrate (NaH_2PO_4, H_2O), and anhydrous di-sodium hydrogen phosphate (Na_2HPO_4) were obtained from Merck (Darmstadt, Germany). Sodium chloride (NaCl) was provided by UCB (Brussels, Belgium). Pesticides standards used for the validation were provided by Dr. Ehrenstorfer (Augsburg, Germany) with the mix 13 (p,p′-DDE, p,p′-DDT, p,p′-DDD, dieldrin, β-endosulfan, γ-HCH, and β-HCH) and the mix 114 (cypermethrin and permethrin), and the remaining standards were supplied from Sigma-Aldrich with purities higher than 95 % except for permethrin and cypermethrin (94 % both). Stable isotope labeled internal standards were purchased from Dr. Ehrenstorfer, Cambridge Isotope Laboratories (Tewksbury, MA, USA) and US Biological (Swampscott, MA, USA).

Preparation of reagents

Stock solutions at 1 g/L in acetonitrile were prepared for each compound. Two sets of working solutions at 0.1, 1, 10, 100, and 1000 μg/L were prepared in acetonitrile, for the quantification of the parent compounds and for their metabolites which required two different preparation procedures. Internal standards (stable isotope labeled analogues) solutions were prepared at 0.1 or 1 mg/L depending on the compound and on the matrix analyzed. Internal standards used for the quantification of pesticide metabolites were as follows: pentachlorophenol-$^{13}C_6$ (PCP-$^{13}C_6$), trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid-D_6 (*trans*-Cl_2CA-D_6), diethyl thiophosphate-D_{10} (DETP-D_{10}), and 3-phenoxybenzoic acid-$^{13}C_6$ (3-PBA-$^{13}C_6$). Internal standards used for parent pesticides were as follows: β-endosulfan-D_4, trifluralin-D_{14}, α-HCH-D_6, γ-HCH-D_6,

trans-permethrin-D_6, p,p′-DDE-D_8, p,p′-DDT-D_8, and diflufenican-D_3. Compounds for which stable isotope labeled analogue was not available were quantified using the best fitted internal standard: DETP-D_{10} was used to quantify DEP and $ClCF_3CA$, α-HCH-D_6 was used to quantify β-HCH, γ-HCH-D_6 was used to quantify fipronil and fipronil sulfone, *trans*-permethrin-D_6 was used to quantify cyhalothrin and cypermethrin, p,p′-DDE-D_8 was used to quantify p,p′-DDD, dieldrin, and oxadiazon.

Instrumentation and GC-MS/MS conditions

Agitation steps were achieved with a KS 15 B horizontal shaker (VWR, Leuven, Belgium) and with a New Brunswick G25 incubator shaker. A Sigma 4-16KS centrifuge and a Sigma 1-16K centrifuge were used for sample centrifugations. Hair pulverization was carried out for 5 min at full speed using a Retsch ball mill apparatus.

The analysis were performed with an Agilent 7890 gas chromatograph system equipped with a HP-5MS capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness) coupled to an Agilent CTC Pal autosampler and to an Agilent 7000A triple quadrupole mass spectrometer operating in negative chemical ionization mode with methane as chemical reagent gas.

The injection was done into the gas chromatography tandem mass spectrometry (GC-MS/MS) system with the pulsed splitless injection mode at a pulse pressure of 35 psi. The quench gas in the collision cell and the collision gas used were helium and nitrogen, respectively. The helium carrier gas flow and the methane used as CI reagent gas at a flow of 40 % were set at 1.2 and 2 mL/min, respectively. Temperature of the injector, the transfer line, and the source was set at 260, 250, and 150 °C, respectively. The temperature program was the same as the one detailed by Hardy and coworkers [15].

For each compound, retention time, monitored transitions, and collision energy were determined using standards (Table S1, Electronic Supplementary Material (ESM)). At least two transitions (one for quantification and one for confirmation) were used to characterize each compound.

Sample preparation procedures

Analysis of parent pesticides and their metabolites in hair

Hair analysis was performed as previously described by Hardy and coworkers [15]. Briefly, after decontamination with water and acetonitrile, a hair strand was pulverized. Then 50 mg of hair powder was weighed, 10 μL of internal standard solution were added, and the hair was incubated overnight at 40 °C in 1 mL of acetonitrile/water (80:20, *v*/*v*). The extract was vortexed and centrifuged, 300 μL of the supernatant was dedicated to parent pesticides analysis by solid-phase microextraction (SPME), and 300 μL was used for metabolites by direct injection.

Regarding parent compound analysis, 7.6 mL of phosphate buffer at pH 7 (1 M) was added to the 300 μL extract and the sample was analyzed with a direct immersion SPME (fiber exposure at 60 °C for 80 min) followed by desorption in the GC injector at 260 °C for 10 min.

Regarding metabolite analysis, the 300 μL extract was evaporated and the residue was derivatized with 100 μL of PFBBr/acetonitrile (1:3, *v*/*v*), 30 mg of potassium carbonate, and 1 mL of acetonitrile for 30 min at 80 °C. Excess PFBBr was removed under a stream of nitrogen at 37 °C and reconstituted in 20 μL of ethyl acetate prior to GC-MS/MS analysis where 2 μL was injected into the system.

Plasma analysis was adapted from urine analysis as previously described [15].

Analysis of parent pesticides in plasma

Rat plasma sample (200 μL) was added to 5 μL of internal standard solution and 400 μL of cold methanol (−20 °C). After adding 1.6 mL of phosphate buffer at pH 7 (1 M), two successive liquid-liquid extractions with 1 mL of a mix of acetonitrile/cyclohexane/ethyl acetate (1:1:1, *v*/*v*/*v*) were achieved. The two organic phases were recombined and evaporated to dryness under a nitrogen stream at 37 °C. The residue was reconstituted in 200 μL of acetonitrile and 7.6 mL of phosphate buffer at pH 7 (1 M) was added. The sample was then analyzed with a direct immersion SPME (fiber exposure at 60 °C for 80 min) followed by desorption in the GC injector at 260 °C for 10 min prior to GC-MS/MS analysis.

Analysis of pesticide metabolites in plasma

Rat plasma sample (50 μL) was hydrolyzed with 100 μL of concentrated hydrochloric acid (32 %) after the addition of 5 μL of internal standard solution and 450 μL of water. The sample was incubated at 90 °C for 1 h and cooled to room temperature. Then 500 mg of sodium chloride was added to the sample and two successive liquid-liquid extractions with 1 mL of acetonitrile/cyclohexane/ethyl acetate (1:1:1, *v*/*v*/*v*) were achieved. After centrifugation, the two organic phases were combined and evaporated to dryness under nitrogen stream at 37 °C. The extract was derivatized by adding 30 mg of potassium carbonate, 1 mL of acetonitrile, and 100 μL of PFBBr/acetonitrile (1:3, *v*/*v*) then incubated at 80 °C for 30 min. Thereafter, the extract was evaporated to dryness. An additional purification was done with a double liquid-liquid extraction by adding 1 mL of a phosphate buffer at pH 7 (0.5 M) and 1 mL of *n*-hexane. The two organic layers of *n*-hexane were then recombined and dried up

under a nitrogen stream. Finally, the residue was reconstituted with 20 μL of ethyl acetate, transfered in an insert, and 2 μL was injected into the system.

Validation parameters

Validation parameters concerning hair analysis were previously presented by Hardy and coworkers [15]. Briefly, 10 concentration levels were prepared with five replicates at each level for both parents and metabolites. The calibration curves ranged from 0.02–8 pg/mg (depending on pesticides) up to 100 pg/mg. Recovery was evaluated at three spiked levels with five replicates: blank samples were spiked at the beginning of the sample preparation and compared to blank samples fortified just before GC-MS/MS analysis for parent pesticides, and just before derivatization in the case of pesticide metabolites.

Concerning plasma analysis, the model used was a simple linear regression with a weighting factor of $(1/x)$. The linearity was assessed by the coefficient of determination, the calibration range depended on the compounds: from 10 to 10,000 pg/mL for all compounds analyzed by SPME (parents); from 100 to 1,500,000 pg/mL for 3-PBA, TCPy, DEP, and DETP; and from 10 to 150,000 pg/mL for all others compounds analyzed after derivatization. Five replicates were done to evaluate intraday precision and accuracy of each level. Limits of quantification (LOQ) were determined as the lowest concentration level with acceptable accuracy and precision (percentage of the target and relative standard deviation within 25 %). Replicates spiked at the end of the sample preparation were also done to evaluate recovery for three levels by comparing samples and blank samples fortified of the same amount just before GC-MS/MS analysis for parent pesticides, and just before derivatization in the case of pesticide metabolites. Parameters for the plasma analysis were presented on Table 1.

Animal experiment

A relevant number of *Long-Evans* female rats ($n=64$) were administered with a mix of pesticides (parents) by gavage, three times a week over a 3-month period. Seven different levels (ranging from 0.004 to 0.4 mg/kg body weight) were applied plus a control group (receiving the vehicle only). Each group, corresponding to one level of exposure, consisted in eight animals. Before the experiment, the backs of rats were shaved in order to ensure that hair collected at the end of the experiment have entirely grown during the experiment. At the end of the experiment, hair was collected by shaving and blood was sampled at sacrifice (4 h after the last gavage) and turned into plasma with EDTA tubes. Analysis was carried out on white hair. All the procedures applied in this study were in compliance with the rules provided by the European Communities Council Directive of 22 September 2010 (2010/63/EU) and authorized by the Ministry of Agriculture, Grand-Duchy of Luxembourg.

Data analysis

Association between plasma and hair pesticide concentrations was studied on the basis of the R_{Pearson} values, using SigmaPlot software (12.5). The identification of "outliers," defined as pesticides or metabolites which presented marginal behavior regarding the correlation between hair and plasma concentrations, was conducted through a "stepwise" approach. Correlation between plasma and hair concentrations was firstly analyzed on all the compounds ($n=23$) for a same level of exposure. Each compound was then successively removed (reduced to $n=22$ compounds) in order to assess the influence on the R_{Pearson} and P values of the different compounds. The outlier behavior of a compound was then formalized by the fact that removing it from the correlation increased significantly the association between hair and plasma concentrations. Once the first outlier was identified, the statistical analysis was conducted a second time to identify possible additional outlier. The process was then reiterated to find other possible outliers. The results of the stepwise approach were displayed in Table 2.

Physicochemical parameters

Values of water solubility, octanol/water partition coefficient (Kow), and acidity constant (pKa) have been obtained from international databases [16–18] and are presented in Table 3, as well as compound molecular weight.

Results and discussion

Validation parameters

Validation parameters for hair analysis were previously detailed by Hardy and coworkers [15]. Briefly, the linearity was assessed by the coefficient of determination, which was always higher than 0.94 and higher than 0.99 for most compounds. LOQs ranged from 0.02 pg/mg for trifluralin to 5.5 pg/mg for DEP. Recovery was within 60–140 % for all the compounds. Parameters for plasma analysis are presented in Table 1. The linearity was assessed by the coefficient of determination, which was always higher than 0.94. LOQs ranged from 10 pg/mL for diflufenican and cyhalothrin to 100,000 pg/mL for DEP. Accuracy and variability were expressed as the percentage of target value and the relative standard deviation (RSD) for each level. For the validated levels, variability was most of the time below 20 % and always below 25 % (as defined for LOQ determination) and accuracy ranged

Table 1 Validation parameters of the plasma analysis

			LOQ	Accuracy (% of target) ($n=5$)										Variability[a] (%) ($n=5$)										Recovery (%) ($n=5$)		
Pesticides	slope	R^2	pg/mL	lev.1	lev.2	lev.3	lev.4	lev.5	lev.6	lev.7	lev.8	lev.9	lev.10	lev.1	lev.2	lev.3	lev.4	lev.5	lev.6	lev.7	lev.8	lev.9	lev.10	A	B	C
Trifluraline[b, c]	1.1	0.986	250	*−67*	*24*	*34*	*57*	83	87	85	96	95	102	22	25	13	18	17	16	20	15	11	7	89	83	89
b-HCH[b, c]	0.22	0.974	500	*769*	*85*	*57*	*31*	*17*	90	76	107	86	104	*70*	*77*	*63*	*50*	*102*	19	23	19	7	8	*110*	96	95
g-HCH[b, c]	0.67	0.989	50	*49*	*68*	90	89	106	104	101	112	96	98	16	14	8	11	12	2	10	13	10	8	98	96	95
Fipronil[b, c]	2.8	0.981	100	*351*	85	110	103	100	87	80	102	94	101	*66*	*48*	*36*	17	12	15	11	9	16	11	95	99	97
p.p′-DDE[b, c]	0.93	0.992	250	*−833*	*−237*	*−51*	*3*	94	89	85	98	98	100	*66*	*53*	*34*	*31*	9	15	9	10	8	6	96	97	94
Dieldrin[b, c]	4.5	0.943	250	*5*	*−4*	*30*	*76*	88	103	79	90	97	104	*88*	*63*	*18*	*43*	28	22	21	24	24	22	101	96	97
Oxadiazon[b, c]	5.6	0.973	100	*−42*	*11*	*48*	85	83	83	74	91	99	104	*43*	*55*	*33*	20	24	23	21	14	16	14	97	84	79
Fipronil sulfone[b, c]	4.2	0.969	50	*84*	*32*	104	87	91	76	77	101	95	104	*45*	15	24	24	8	14	19	17	20	16	97	96	97
Endosulfan-beta[b, c]	0.71	0.989	25	*74*	96	93	94	113	107	98	98	*85*	*69*	*27*	19	18	11	11	7	10	9	4	3	92	99	96
p.p′-DDD[b, c]	0.67	0.981	10^3	*9675*	*2630*	*3480*	*1720*	*670*	*285*	77	94	89	100	*75*	*117*	*23*	*19*	*47*	*77*	15	12	5	10	*297*	87	85
p.p′-DDT[b, c]	0.81	0.965	500	*3092*	*1399*	*903*	*816*	*136*	114	81	68	74	106	*141*	*82*	*110*	*66*	*115*	17	15	27	17	11	*72*	69	70
Diflufenican[b, c]	1.8	0.991	10	112	104	86	77	88	87	82	96	98	105	17	6	15	13	9	17	7	6	8	8	99	94	93
Cyhalothrine[b, c]	45	0.992	10	74	97	88	79	98	97	95	96	99	102	29	27	11	8	10	16	14	15	11	3	91	83	83
Permethrine[b, c]	6.8	0.987	100	*224*	*149*	*55*	83	102	85	83	105	97	101	*110*	13	14	24	23	15	18	18	10	7	94	91	92
Cypermethrine[b, c]	130	0.989	50	*61*	*51*	78	76	93	103	89	99	97	102	*61*	*50*	16	21	9	13	15	12	15	6	94	91	94
DEP[d, e]	0.27	0.982	10^5	*5*	*−30*	*−16*	*26*	*53*	*61*	74	93	101	103	*21*	*25*	*14*	*42*	*44*	*30*	14	20	5	9	*56*	54	60
ClCF3CA[d, f]	0.14	0.969	10^3	*−553*	*−91*	*−18*	*48*	72	82	76	94	93	108	*28*	*51*	*22*	*29*	28	23	25	9	36	12	47	46	47
DETP[d, e]	1.29	0.991	4.10^3	*23*	*48*	*48*	81	82	94	80	89	105	101	*34*	*42*	*26*	6	7	4	7	9	7	7	75	75	75
Carbofuranphenol[d, f]	0.19	0.973	10^3	*−3303*	*−806*	*−265*	*−14*	88	80	116	92	102	103	*51*	*58*	*65*	*43*	23	11	12	25	15	14	51	50	51
TCPY[d, e]	0.61	0.981	4.10^3	*−734*	*−234*	*−2*	73	114	121	125	119	93	99	*89*	*36*	*65*	8	14	14	5	7	18	3	77	77	82
PCP[d, f]	1.9	0.995	400	*−608*	*−170*	*−2*	81	89	93	96	92	103	100	9	10	10	7	6	8	5	7	8	5	89	71	74
3-PBA[d, e]	2.3	0.998	10^3	*31*	*38*	72	89	97	98	93	95	102	101	24	5	5	5	4	2	8	6	2	4	84	80	81
Cl2CA[d, f]	1.2	0.996	400	*−553*	*−99*	*1*	75	89	94	94	93	101	102	11	20	11	7	6	2	5	7	6	6	72	73	74

Values in italic are below criteria of validation, these levels were not validated

[a] Variability is expressed by RSD

[b] Analyzed by SPME

[c] Level 1 was 10 pg/mL and level 10 was 10,000 pg/mL, for the recovery level A was 1000 pg/mL, level B was 2500 pg/mL, and level C was 10,000 pg/mL

[d] Analyzed after derivatization

[e] Level 1 was 100 pg/mL and level 10 was 1,500,000 pg/mL, for the recovery level A was 10,000 pg/mL, level B was 100,000 pg/mL, and level C was 1,000,000 pg/mL; values in color are below criteria of the validation, these levels were not validated

[f] Level 1 was 10 pg/mL and level 10 was 150,000 pg/mL, for the recovery level A was 1000 pg/mL, level B was 10,000 pg/mL, and level C was 100,000 pg/mL

Table 2 Stepwise statistical analysis of the association between hair and plasma concentration

	All compounds		All without PCP		All without PCP and 3-PBA		All without PCP, 3-PBA and β-HCH		All without PCP, 3-PBA, β-HCH, dieldrin, and ClCF3CA	
Compound removed from the model	$R_{Pearson}$	*P* value	$R_{Pearson}$	*P* value	$R_{Pearson}$	*P* value	$R_{Pearson}$	*P* value	$R_{Pearson}$	*P* value
None	0.284	0.000262	0.525	2.74E−12	0.736	2.28E−26	0.875	2.97E−45	0.893	9.12E−45
Beta-HCH	0.383	9.71E−07	0.630	1.22E−17	*0.875*	*2.97E−45*				
Gamma-HCH	0.286	3.26E−04	0.529	5.60E−12	0.740	1.60E−25	0.883	6.27E−45		
p,p′-DDE	0.287	3.14E−04	0.514	2.83E−11	0.732	9.70E−25	0.877	1.64E−43		
p,p′-DDD	0.277	5.11E−04	0.519	1.73E−11	0.732	8.28E−25	0.873	1.03E−42		
p,p′-DDT	0.287	3.13E−04	0.526	7.71E−12	0.738	2.34E−25	0.879	7.45E−44		
Dieldrin	0.294	2.17E−04	0.516	2.24E−11	0.729	1.75E−24	*0.894*	*1.48E−47*		
Beta-endosulfan	0.275	5.68E−04	0.516	2.34E−11	0.731	1.23E−24	0.872	1.74E−42		
PCP	*0.525*	*2.74E−12*								
DEP	0.276	5.22E−04	0.518	1.80E−11	0.733	8.06E−25	0.873	9.17E−43		
DETP	0.276	5.23E−04	0.518	1.84E−11	0.732	8.93E−25	0.873	1.14E−42		
TCPY	0.285	3.34E−04	0.527	6.77E−12	0.743	7.74E−26	0.882	9.98E−45		
Cyhalothrin	0.278	4.73E−04	0.523	1.08E−11	0.739	1.85E−25	0.881	2.63E−44		
Permethrin	0.274	5.72E−04	0.515	2.40E−11	0.731	1.26E−24	0.872	1.79E−42		
Cypermethrin	0.275	5.62E−04	0.516	2.26E−11	0.731	1.16E−24	0.872	1.55E−42		
Cl2CA	0.284	3.49E−04	0.534	3.21E−12	0.756	3.45E−27	*0.895*	*1.04E−47*		
ClCF3CA	0.276	5.39E−04	0.517	1.99E−11	0.732	9.69E−25	0.873	1.25E−42		
3-PBA	0.312	8.09E−05	*0.736*	*2.28E−26*						
Trifluraline	0.275	5.69E−04	0.516	2.35E−11	0.731	1.24E−24	0.872	1.75E−42		
Oxadiazon	0.277	5.14E−04	0.519	1.70E−11	0.733	7.23E−25	0.874	7.64E−43		
Fipronil	0.278	4.90E−04	0.520	1.51E−11	0.734	6.27E−25	0.874	7.07E−43		
Fipronil sulfone	0.226	4.76E−03	0.324	6.11E−05	0.668	2.12E−19	0.766	7.36E−27		
Diflufenican	0.277	5.06E−04	0.519	1.68E−11	0.733	7.95E−25	0.873	9.26E−42		
Carbofuran phenol	0.280	4.41E−04	0.523	1.08E−11	0.736	3.50E−25	0.879	7.10E−44		

Values in italics showed the highest increase of both *P* value and $R_{Pearson}$ when they were removed

from 75 to 125 %. Nevertheless, level 5 of $ClCF_3CA$ which corresponded to its LOQ was validated with an accuracy of 73 % and a variability of 28 %. For the majority of the compounds, recovery was within the range of 70–100 % with the exception of DEP, $ClCF_3CA$, and carbofuran phenol for which it was close to 50 %. The low recovery of carbofuran phenol might be explained by its high water solubility (1096 mg/L) as displayed in Table 3.

Several studies have been conducted on the detection of pesticides in blood. Concerning organochlorines, two population studies reported concentrations compatible with the range of the method presented here. Investigating exposure of Mexican sub-groups, Ruiz-Suarez and coworkers detected β-HCH, γ-HCH, p,p′-DDE, p,p′-DDT, and β-endosulfan in plasma with LOQ between 1780 and 3790 pg/mL [19], which is between 10 to 35 higher than the sensitivity of the present method. Curren et al., investigating exposure of Canadian women, reported plasma concentration ranging from 100 to 260 pg/mL for γ-HCH [20] which would be compatible with the performance of the method presented here (validated range: 50 to 10,000 pg/mL). On the contrary, Wittsiepe and coworkers validated a method to determine β-HCH in serum with a LOQ three times lower than the present study [21]. This good sensitivity has however to be balanced by the fact that the method was specific to organochlorine compounds only. As pointed out by Appenzeller and Tsatsakis, the sensitivity of an analytical method is clearly affected by its specificity [22]. The sensitivity of the method presented here can therefore be considered quite satisfactory regarding the number of different chemical classes it covers. Pyrethroid metabolites such as 3-PBA and Cl_2CA were also already quantified in human plasma and serum [23, 24], but with LOQ three to five times higher than in the present work for 3-PBA and

Table 3 Physicochemical parameters of the target compounds

	Molecular weight (g/mol)	Water solubility (mg/L)	Log Kow[a]	pKa
Organochlorines				
β-HCH[b]	*290.8*	*0.24*	*3.78*	
γ-HCH	290.8	7.3 [10]/8.5 [8]	3.72 [10]/3.5 [9]	
p,p′-DDE	318	0.04	6.51	
p,p′-DDD	320	0.09	6.79	
p,p′-DDT	354.5	0.0055	6.91	
Dieldrin	380.9	0.19[10]/0.14[9]	3.7	
β-Endosulfan	406.9	0.32	4.75	
PCP[b]	*266.3*	*14*	*5.12* [10]/*3.32* [9]	*4.7*
Organophosphates and their metabolites				
DEP	154.1			
DETP	170.2			2.9
TCPy	198.4	80.9	3.21	
Pyrethroids and their metabolites				
Cyhalothrin	449.9	0.003	6.9	
Permethrin	391.3	0.006	6.5	
Cypermethrin	416.3	0.004 [8]/0.009 [9]	6.6 [8]/5.3[9]	
Cl_2CA	209.1		3.2	
ClCF3CA	242.6			
3-PBA[b]	*214.2*	*16.9*	*3.91*	*3.9*
Miscellaneous pesticides				
Trifluralin	335.3	0.22	5.27	
Oxadiazon	345.2	0.7	5.33	
Fipronil	437.1	1.9	3.75	0.24
Fipronil sulfone	453.1			
Diflufenican	394.3	0.05		
Carbofuran phenol	164.2	1096	2.08	

[a] Octanol/water partition coefficient (log Kow)

[b] Outliers are highlighted in italic characters

up to 10 times higher for Cl_2CA. Concerning phenylpyrazoles, the present method presented LOQs of 100 and 50 pg/mL for fipronil and fipronil sulfone respectively, while in the literature, Moser and coworkers determined LOQs of 10,000 pg/mL for both compounds in an experiment of controlled exposed rats [25].

Regarding plasma concentration reported in the literature for different pesticides, the performance of the method presented here should therefore be adequate to determine human exposure in the future.

Association between plasma and hair concentration

The distribution of plasma versus hair concentrations of the target chemicals in animals for all the levels of exposure was presented on Fig. 1A. A visual examination of the figure showed a general trend of the association between hairs and plasma confirmed by the statistical analysis (Pearson correlation *P* value, 2.55E−49) although the trendline (linear fit) presented poor R^2 value (0.12). As detailed by Appenzeller et al., the number of different target chemicals detected in both hair and plasma increased with increasing level of exposure [26]. The association between hair and plasma concentrations was therefore investigated using the data from the highest level of exposure group (corresponding to a dose of 0.4 mg/kg b. w.) in order to have the largest possible number of compounds. The distribution of plasma versus hair concentrations of the target chemicals in animals exposed to the highest level of exposure is presented on Fig. 2. A first visual examination of the graphs supported the association between hair and plasma concentrations for most compounds, but also suggested that some outliers behaved in different ways by deviating from the general trend. This phenomenon was clearly highlighted with linear scale for PCP, 3-PBA, and β-HCH (Fig. 2A). The outlier behavior of these compounds was confirmed by the statistical stepwise analysis (Table 2). The correlation coefficient

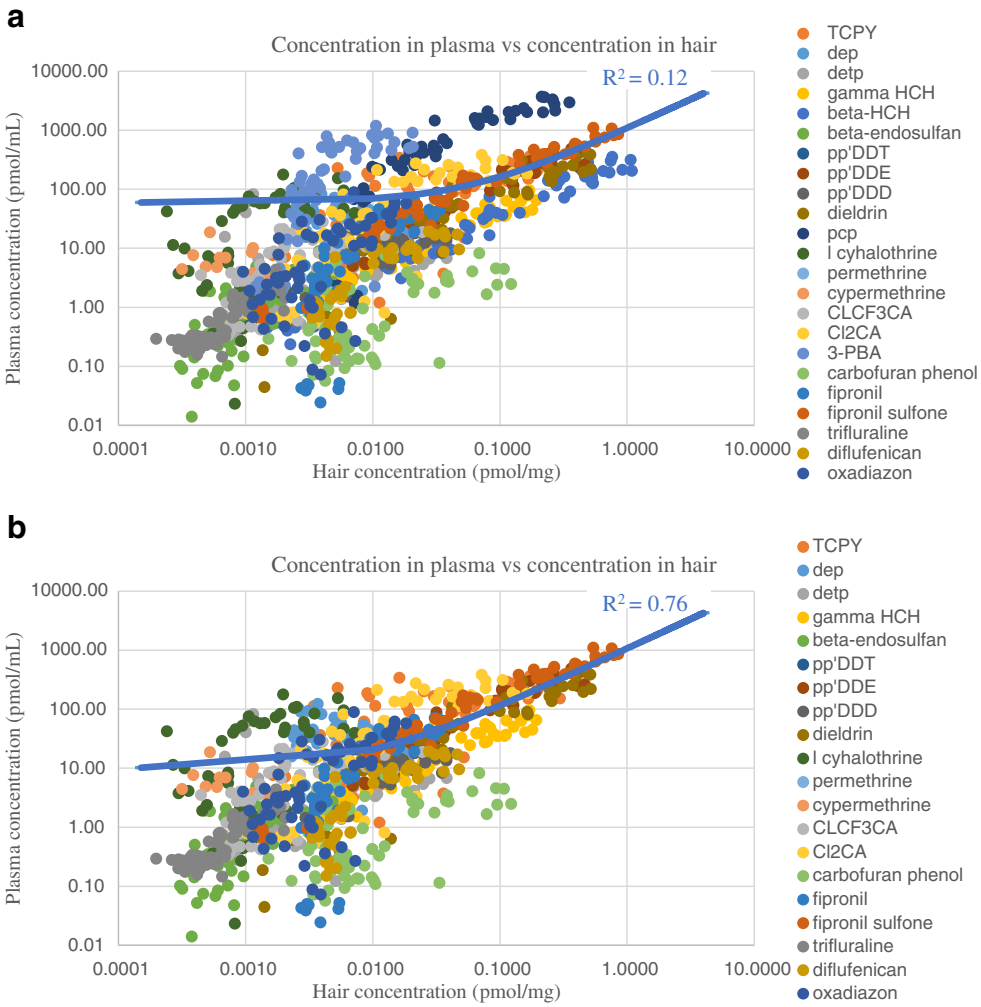

Fig. 1 Plasma versus hair concentration of the target chemicals in animals from all levels of exposure group presented in logarithmic scale (**A**) and without the three outliers (**B**): PCP, 3-PBA, and β-HCH. Each compound was identified by one color and the *line* represented the trendline of the distribution

associated with the linear regression including the 23 compounds was 0.284 and the *P* value was 0.000262. PCP was identified as the main outlier because removing this compound from the correlation increased the *R* value to 0.525 and the *P* value to 2.74E−12. 3-PBA and β-HCH were also considered outliers since removing the first one increased the correlation coefficient to 0.736 and the *P* value to 2.28E−26 and removing the two compounds increased the *R* value to 0.875 and the *P* value to 2.97E−45. Since no other compound removal allowed significant increase in *R* and *P* values, only PCP, 3-PBA, and β-HCH were considered outliers. When the three outliers were removed from the model for all the levels of exposure (Fig. 1B), the trendline was more adapted to all the data in comparison with the trendline presented in Fig. 1A as the R^2 increased from 0.12 to 0.76. These results confirmed that only PCP, 3-PBA, and β-HCH had to be considered outliers. For 20 out of the 23 compounds, the concentration in hairs was proportional to the concentration in plasma with quite similar plasma to hair ratio. The plasma to hair ratio was higher for PCP and 3-PBA, suggesting lower incorporation from plasma and, on the contrary, was lower for β-HCH, suggesting higher hair incorporation than the other compounds for equivalent plasma concentration (Fig. 3).

Comparing PCBs and organochlorines concentrations in blood and hair, Altshul et al. observed good correlation between the two matrices for p,p′-DDE ($R_{spearman}$ = 0.8) and low correlation for p,p′-DDT ($R_{spearman}$ = 0.4) which was explained by easy metabolization of p,p′-DDT into p,p′-DDE [8]. In comparison, the present study showed a highly significant correlation for these two compounds with Spearman correlation coefficients of 0.97 for p,p′-DDE and 0.94 for p,p′-DDT with *P* values <0.001.

Physicochemical properties

The values found for water solubility, log Kow, pKa, and molecular weight of all the compounds are presented in Table 3. Some values were not available in the literature for water solubility and log Kow. On the contrary, several different values were found in different databases for the same

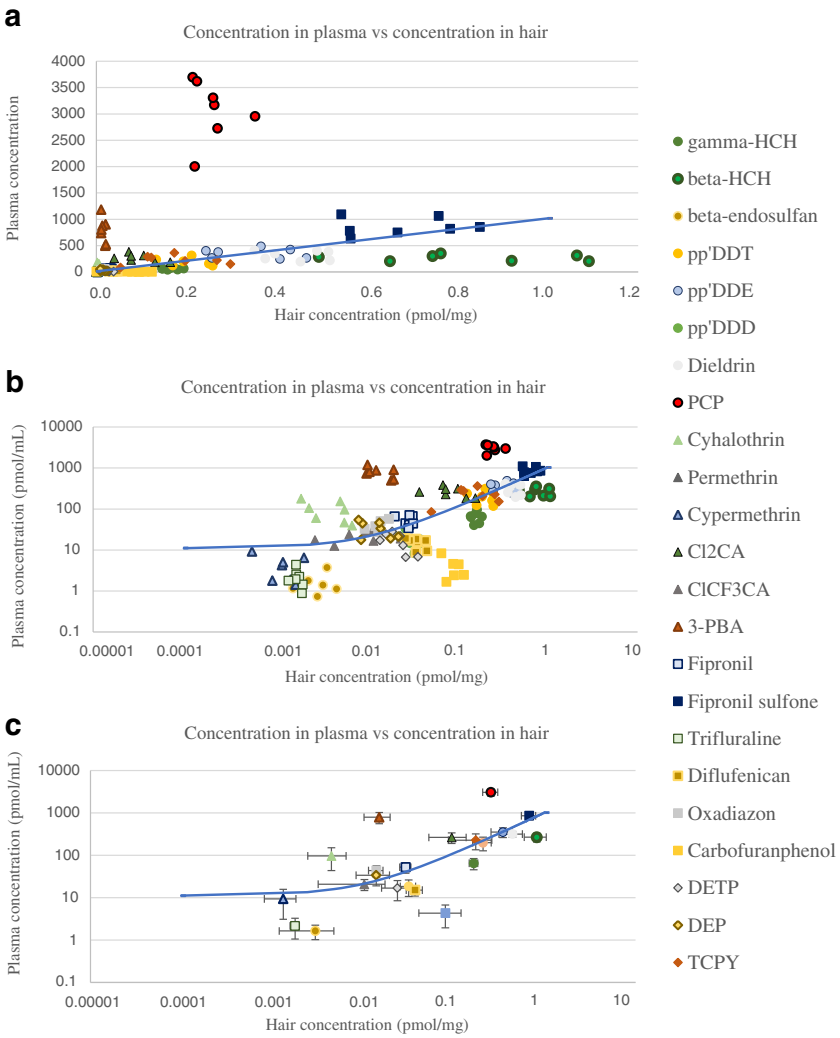

Fig. 2 Plasma versus hair concentration of the target chemicals in animals from the highest level of exposure group, presented in **A** linear scale; **B** logarithmic scale; **C** as average value (with standard deviation) of the seven animals for each chemical. Each compound was identified by one color for each of the seven animals (one rat of the most exposed group died before the end of the experiment)

compound. For instance, water solubility of γ-HCH was estimated at 7.3 or 8.5 mg/L and the log Kow of PCP was 3.32 or 5.12 depending on the consulted database. Since the majority of the compounds do not present acidic properties, pKa values were obtained for only a few compounds. These values were investigated with regard to biologically relevant pH conditions: the one of blood and the one of keratinocytes. To the best of our knowledge, only little research have been carried out on the effect of compound physicochemical properties on their incorporation into hair.

Water solubility

Although two different values were obtained from databases for γ-HCH, dieldrin, and cypermethrin, these values were quite close to each other compared with the other

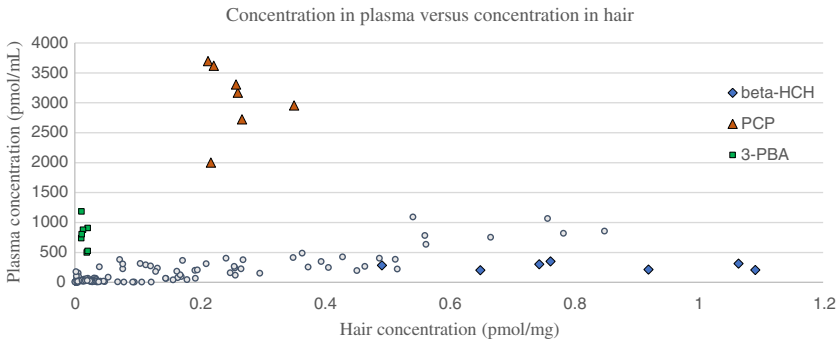

Fig. 3 Distribution of the three outliers in comparison with the others compounds

compounds. This parameter ranged from 0.003 mg/L for cyhalothrin to 1096 mg/L for carbofuran phenol. The water solubility of PCP, β-HCH, and 3-PBA was 14, 0.24, and 17 mg/L, respectively, which does not suggest specific behavior of these compounds at that level.

Although the different behavior of PCP and 3-PBA (higher plasma/hair ratio) compared to β-HCH (lower plasma/hair ratio) is in line with their different solubility, this association is contradicted by TCPy and carbofuran phenol which present normal and even relatively low plasma/hair ratios despite their high solubility in water (80 and 1096 mg/L). Similarly, compounds with lower solubility in water than β-HCH however presented lower plasma/hair ratio (Fig. 1). These results therefore suggest limited or no influence of water solubility on the incorporation into hair. No study was found in the literature on the correlation between water solubility and the incorporation into hair.

Lipophilicity

Lipophilicity was illustrated by the logarithmic value of the octanol/water partition coefficient (log Kow). Although two different values were obtained from databases for three compounds (γ-HCH, PCP, and cypermethrin), these values were sufficiently close, compared with the other compounds, to exclude possible interpretation bias. The log Kow ranged from 2.08 to 6.91 for the different compounds and equaled 3.78 for β-HCH, 3.91 for 3-PBA, and 3.32 or 5.12 for PCP. Lipophilicity therefore seemed not to be a determining criterion in the incorporation of chemicals into hair.

By comparing the ratios "hair concentration" to "plasma AUC" (Area Under Curve "plasma concentration vs time"), Nakahara and coworkers concluded that incorporation rate of amphetaminic derivates into hair was correlated with lipophilicity for 20 drugs of abuse [27]. The lipophilicity was deduced from the retention times of reverse phase high performance liquid chromatography. The correlation between incorporation rate and lipophilicity was very low but rose to a significant value (0.201 to 0.770) when a highly lipophilic drug (log Kow = 7.95) was removed from the model [10, 27, 28]. The main difference with the present study was the use of the AUC values instead of plasmatic concentration 4 h after the exposure.

Molecular weight

Molecular weight ranged from 154 (for DEP) to 453 g/mol (for fipronil sulfone). The molecular weight of the three outliers which were 214, 266, and 291 g/mol, therefore felt in the average range and was unlikely to explain their specific behavior.

Cellular membranes form an impermeable barrier for organic ions of medium molecular mass which could avoid them to diffuse easily into the matrix of hair cells as proposed by Pragst [29]. Kintz suggested that lipophilic compounds below 600 g/mol could easily cross biological membrane due to passive transfer from the more concentrated medium to the less one [14]. Although the range of molecular weight of the compounds investigated in the present work (<500 g/mol) did not allow observing the behavior of high-molecular weight compounds (>600 g/mol), incorporation in hair did not decrease with increasing molecular weight.

Charge

The charge of the compounds was extrapolated from their pKa value with regard to the pH of the biological compartments theoretically involved in the transfer: blood (7.35–7.45) and living hair bulb cells (between 3 and 6) [30]. The majority of the compounds have no acidic properties and therefore remain uncharged irrespective of the pH of the media. Acidic constant values were obtained from databases for four compounds only: PCP, DETP, 3-PBA, and fipronil.

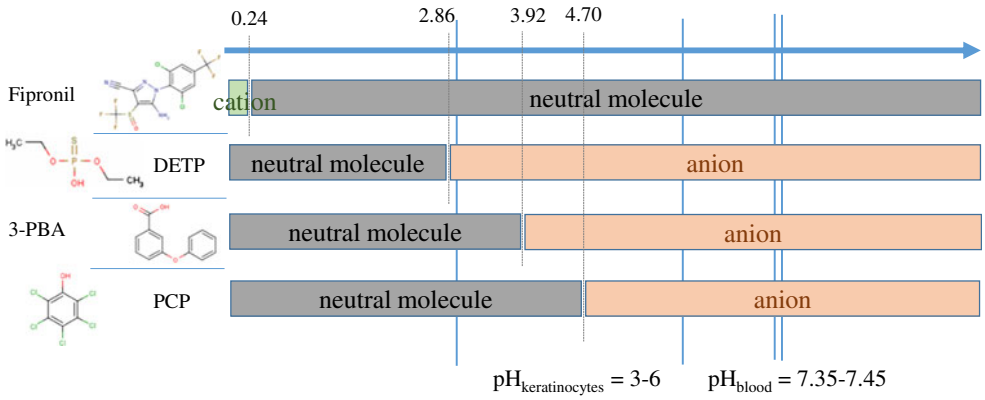

Fig. 4 Charge of fipronil, DETP, 3-PBA, and PCP depending on their pKa and the pH of the system

DETP and fipronil have their pKa below 3 and would thus not be influenced during the pH change when crossing the membrane between blood and hair cells. On the contrary, 3-PBA and PCP have their pKa between the pH of blood and the pH of keratinocytes. They would therefore move from anionic form in blood to neutral in hair (Fig. 4). As the range of pH of keratinocytes is wide, the charge modification of these two compounds cannot be firmly established but remains possible. Such a charge modification would be in line with the divergent behavior of two of the three outliers compared to the other compounds but cannot explain the behavior of β-HCH which remains uncharged whatever the pH.

The influence of charge has already been questioned by Pragst who suggested that the pKa of the compound and the pH of the matrix would play an important role in the accumulation of basic drugs in hair cells matrix [29]. Nakahara and coworkers demonstrated that neutral or acidic drugs were poorly incorporated into hair compared to basic drugs [31] and that incorporation would depend on the structural factors of compounds such as the length of carbon branches at the N-position of amphetamine (increasing incorporation into hair) or the presence of an hydroxyl group (decreasing incorporation into hair)[28]. Kintz suggested that basic drugs may accumulate in keratinocytes, and that once in the hair cell, the compound would be protonated and not able to diffuse back into the plasma [30]. The latter hypotheses can however not be directly transposed to the compounds investigated in the present work due to structural differences.

Conclusion

This study represents the first investigation of the influence of pesticide physicochemical parameters on the relationship between plasma and hair concentration. The results support that the concentration of chemicals in hair depends on the respective concentration in plasma and suggest that for most compounds, the transfer through the cellular membrane at the level of the hair bulb cells would not limit incorporation of chemicals irrespective of their physicochemical properties. Although the behavior of the three outliers was not elucidated yet, this should be further investigated with regard to the pharmacokinetic parameters which could vary greatly between the different compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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