Investigation of background contribution from different solvent brands used for sample preparation and analysis of persistent organic pollutants

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1 Introduction

In the times of pandemic, we experienced problems with supply bottlenecks for the solvents normally used for extraction and analysis of persistent organic pollutants (POPs; i.e. PCBs, PBDEs and organochlorine pesticides) in our laboratory and, considered to start using solvents from another producer alternatively. Since we analyze POPs at very low concentration levels and other consumables are background tested before use, it was decided to investigate solvents from different brands as applied for sample preparation of POPs in our in-house method. Solvents were tested as they were aliquoted out of the bottle together with upconcentrated solvent samples. For the upconcentrated samples the same amount of solvent was used as in the in-house method for extraction of POPs in human serum and plasma samples. The solvents investigated were n-hexane, isooctane, dichloromethane (DCM) and acetone.

2 Materials and Methods

Standards and Chemicals: Organic solvents of SupraSolv GC-MS-grade quality (acetone, DCM and n-hexane) were purchased from Merck (Merck KGaA, Darmstadt, Germany); isooctane was of SupraSolv GC quality. The same four solvents were purchased from Honeywell (Honeywell, Bucharest, Romania), with Chromasolv for Pesticide Residue Analysis quality.
Native and isotope labelled single standards for selected organochlorine pesticides, PCBs and PBDEs were purchased from Cambridge Isotope Laboratory (Andover, MA, USA), Chem Service (West Chester, PA, USA) or Wellington Laboratories (Guelph, Ontario, Canada).Standard stock mixtures were prepared in isooctane and stored in glass vials at <-20°C. Quantification standard mixtures were prepared in isooctane at concentrations ranging from 1.7 to 7 pg/mL for individual isotope labelled compounds and from 0.003 to 10 pg/mL for native compounds. Standard solutions for daily use were stored at 4-8°C.
Sample preparation. 50 µL n-hexane, isooctane and DCM were pipetted directly into GC vials. n-hexane and isooctane were analyzed directly, whereas DCM was evaporated to dryness and dissolved in 50 µL isooctane prior to instrumental analysis. For simulation of the different sample preparation steps an up-concentration for the solvents was done as follows: aliquots of 3160 µL n-hexane were evaporated in 2mL glass vials to approximately 500 µL, then transferred into GC vials with disposable glass Pasteur-pipettes and evaporated to dryness. 50 µL n-hexane was added (sample ID: n-hexane evap. A). The same procedure was executed for 1500 µL n-hexane (sample ID: n-hexane evap. B). For DCM, aliquots of 770 µL were evaporated to dryness directly in the GC vials, and dissolved in 50 µL isooctane. The same procedure as applied for DCM was followed for 160 µL acetone and 70 µL isooctane. Three replicates were prepared for every solvent test procedure. No internal standards were added to the solvent samples in order to avoid contamination of the solvents with native compounds found as impurities in the ^{13}C-labelled internal standards.
Instrumentation and measurements. Gas chromatography atmospheric pressure ionisation coupled to tandem mass spectrometers (GC-API-MS/MS; Waters, Milford, MA, USA) were used for instrumental analysis. The API was conducted in positive mode under charge transfer conditions. For detection on the mass spectrometer, the multiple reaction monitoring mode was applied with two specific transitions for the individual analytes. (Huber, Averina, & Brox) Quantification was performed using the Masslynx and Targetlynx software (Version 4.2, Waters, Milford, MA, USA). An external eight-point calibration curve with concentrations ranging from 0.003 pg/µL to 1 pg/µL was applied for analysis, but due to very low concentrations in the samples only the lowest detected quantification standard, for most components 0.005 pg/µL, was used for a semiquantitative calculation of the concentrations.

3 Results

In isooctane 12 different compounds were detected (Figure 1 and Table 1), whereas there were 11 compounds detected in n-hexane (Figure 2 and Table 2). Eight components were present in both solvents: the PCBs 28, 52, 101, 149 and 153, *p,p'*-DDE, HCB, and γ-HCH. In addition, PCB 180, Aldrin, β-HCH, and Heptachlorepoxide were detected in isooctane and OCS, and the PCBs 118 and 153 in n-hexane. In DCM 8 different compounds could be detected (Figure 3 and Table 3): the PCBs 28, 52, and 149, HCB, α-, γ-, and β-HCH, and Oxychlordane. In acetone only two compounds were detected (Figure 4 and Table 4), namely PCB 28, and γ-HCH. These two compounds are present in all four different solvents. The compound with highest detection frequency was PCB 28 (33 detects out of 48 possible), while the compound found in highest concentrations was HCB (up to 23 fg/µL). However, in many cases not all compounds were detected in all three replicates and the concentrations measured in the replicates were fluctuating due to the very low concentration levels present in the solvents.

Table 1: Number of detects in isooctane. Solvent samples were analysed in triplicates.

	Merck	evap. Merck	Honeywell	evap. Honeywell
PCB 28		3	1	3
PCB 52		1		1
PCB 101		2		
PCB 149		1		
PCB 153		1		
PCB 180		1		
***p,p'*-DDE**		1		
HCB		3		1
Aldrin			1	
γ-HCH		1		1
β-HCH				1
cis-Heptachlorepoxide	1			

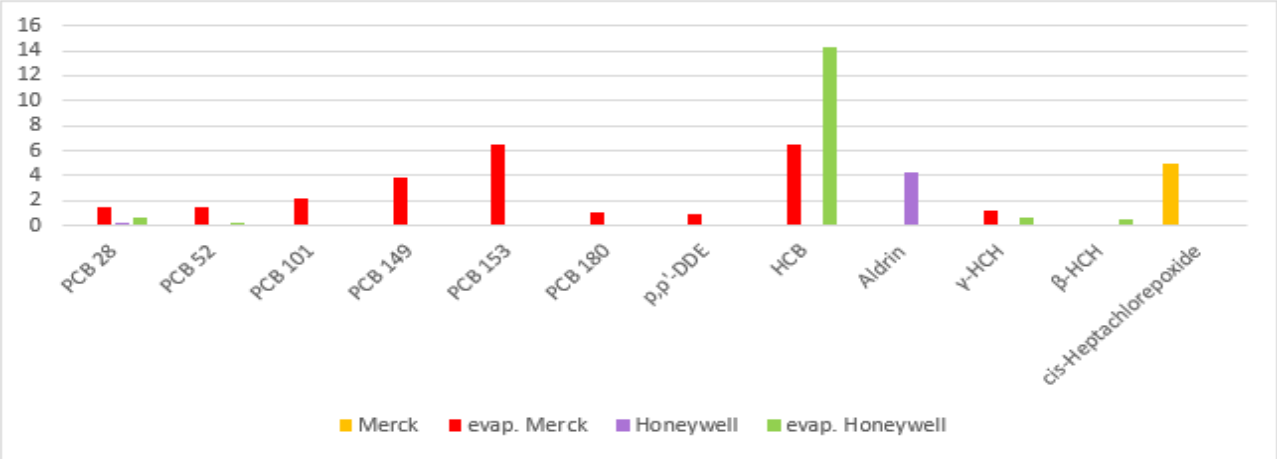

Figure 1: Average concentrations of individual POPs detected in isooctane (fg/µL).

Table 2: Number of detects in n-hexane. Solvent samples were analysed in triplicates.

	Merck	evap. A Merck	evap. B Merck	Honeywell	evap. A Honeywell	evap. B Honeywell
OCS		3	3			
PCB 28	2	3	1	3	3	3
PCB 52		3	2		3	2
PCB 101		2	1		2	2
PCB 118						2
PCB 149		3	3		3	2
PCB 153		3	3		2	2
PCB 138		2	2		1	2
p,p'-DDE		3	1		2	2
HCB	1	3	2		2	2
γ-HCH		2			1	2

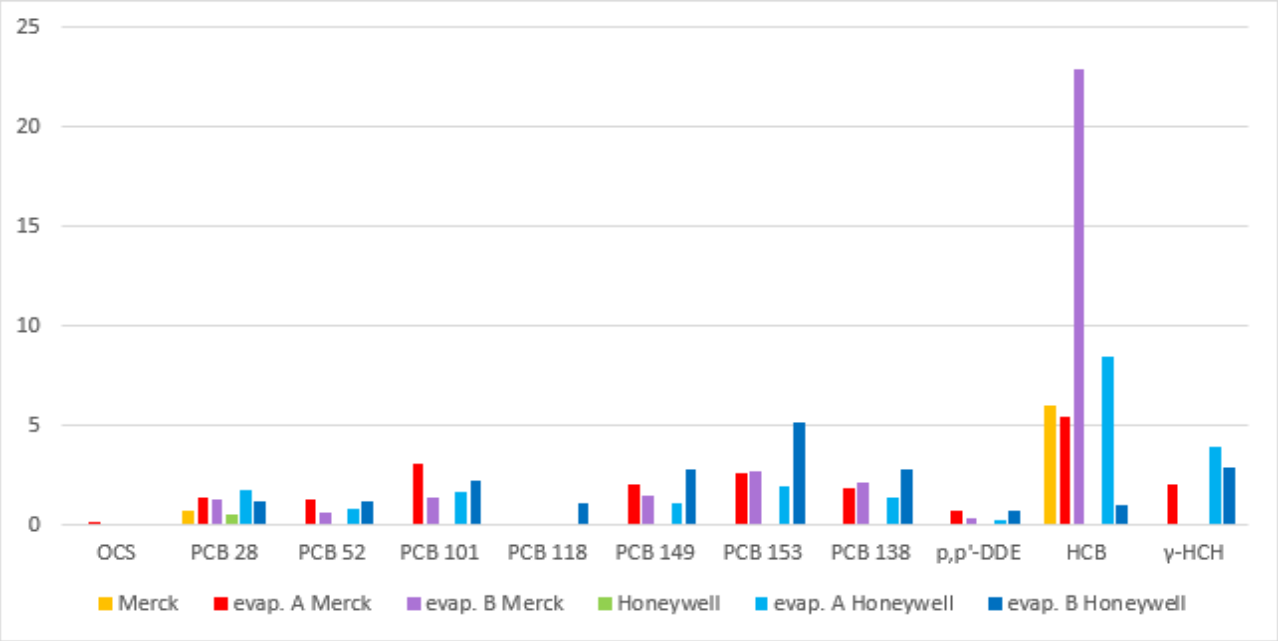

Figure 2: Average concentrations of individual POPs detected in n-hexane (fg/µL).

Table 3: Number of detects in dichloromethane. Solvent samples were analysed in triplicates.

	Merck	evap. Merck	Honeywell	evap. Honeywell
PCB 28	3	1	3	1
PCB 52		1		1
PCB 149		1		
HCB				1
α-HCH	1			
γ-HCH		1		1
β-HCH	1			
Oxychlordane	1			

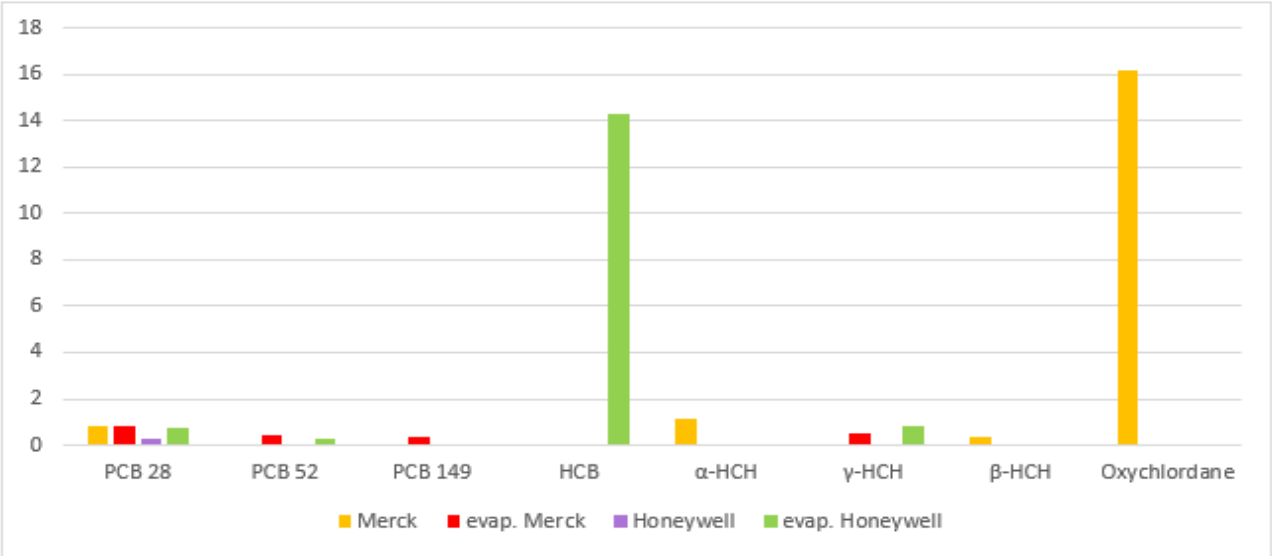

Figure 3: Average concentrations of individual POPs detected in dichloromethane (fg/µL).

Table 4: Number of detects in acetone. Solvent samples were analysed in triplicates.

	evap. Merck	evap. Honeywell
PCB 28	2	1
γ-HCH	1	

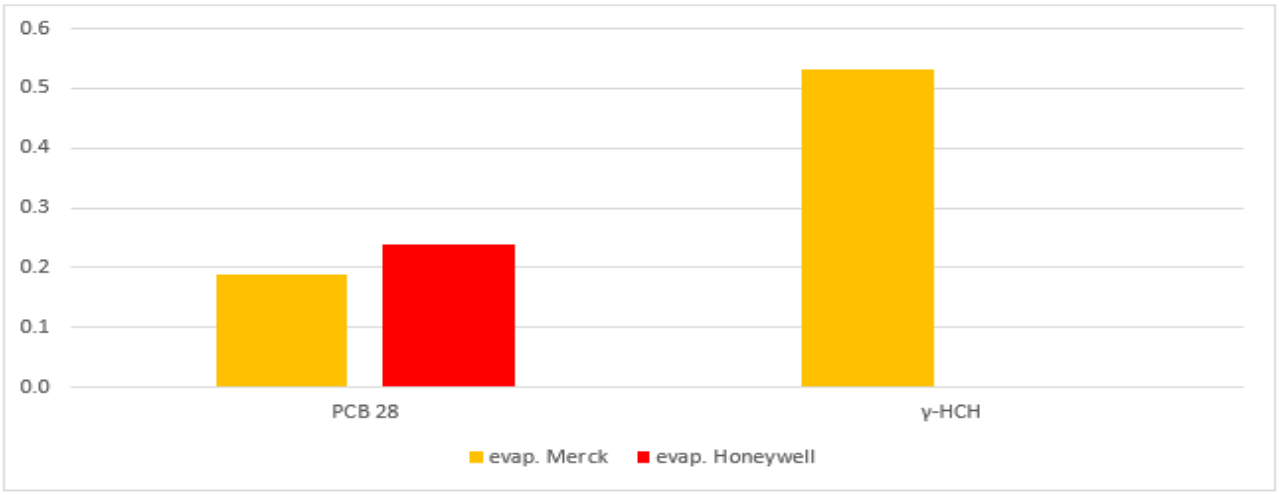

Figure 4: Average concentrations of individual POPs detected in acetone (fg/µL).

4 Discussion and conclusions

The solvents from both analysed brands have some background contamination. There are some differences between the two brands, depending on the solvent and the compounds, but the differences are very small. Thus, it is not possible to give a clear recommendation for preferences on using one solvent brand over the other.

As mentioned earlier, we used an external calibration curve, which may be a limitation for calculating the resulting concentrations. But this was an elaborate decision in order to prevent introducing possible and minor background contribution coming from impurities of the isotope labelled standards, to avoid misinterpretation/false positive detects.

Anyway, the results indicate that there are some POPs present in the organic solvents tested in this study and that solvents have to be chosen carefully also in relation to the compounds that are going to be analyzed.

In the present study only one batch of solvents was investigated for background contamination. In the future, an additional check of the batch to batch variability is recommended, which will give a better indication of variability in background contamination. In order to increase accuracy of POPs measurements in solvents, repetitive measurements with several replicates should be done with higher volumes of solvents.

To our best knowledge there are no previous published studies focusing on concentration levels of POPs in different brands of organic solvents used for extraction and sample preparation. This information is of interest for especially those who analyze legacy POPs at very low concentration levels. And since the compounds analyzed are mostly banned or restricted in use, the concentrations in human and environmental samples will drop further over the next years. Therefore, it is and will be still more crucial to have a very low background contribution from solvents during sample preparation and analysis to get adequate detection limits.

5 Acknowledgments

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6 References

Huber, S.; Averina, M.; Brox, J. Automated sample preparation and GC-API-MS/MS as a powerful tool for analysis of legacy POPs in human serum and plasma. Anal. Methods **2020**, 12, 912–929.