Improved automated sample preparation for dioxins using parallel gas assisted accelerated solvent extraction with inline automated solvent concentration

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Abstract

Purpose: To demonstrate a method for the determination of dioxins and furans (DxF) in soils using the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor, a newly developed and fully automated parallel extraction and evaporation system.

Methods. This work will demonstrate the advancements of an analytical method to determine dioxins in various soil matrices using a fully automated solvent extraction and in-line concentration system for the determination of 15 PCDDs and PCDFs.

Results: This accelerated solvent extraction method proved to be simple to implement and much faster and more productive than the ASE 350 workflow. The system was able to minimize solvent consumption using gas assisted accelerated solvent extraction which results in faster concentration times greater sample throughput. The EXTREVA ASE washing function was able to carry out solvent washing in between samples throughout the sample extract flow paths. This is a critical step when working with Dioxins and proved effective to not have any reportable carryover in between samples.

Introduction

The EXTREVA ASE Accelerated Solvent Extractor system (Figure 1) is an advanced sample preparation system that incorporates proprietary technologies[1,2]. including gas-assisted dynamic accelerated solvent extraction and parallel accelerated solvent extraction. This fully automated system combines extraction and evaporation functions in a single instrument, ensuring sample integrity through a closed system design. Traditional methods like Soxhlet, sonication, and microwave extraction are commonly used for extracting dioxins and PCBs from soil. However, these methods are labor-intensive, require large volumes of solvents, and may extract unwanted compounds alongside the target analytes. As a result, they can yield subpar analytical results and lead to increased maintenance costs due to nonvolatile material buildup in the GC injection port and column. To address these challenges and reduce solvent usage in the preparation of solid, soil, and sediment samples, accelerated solvent extraction (U.S. EPA Method 3545A) was developed. The EXTREVA ASE system leverages gas-assisted solvent delivery and parallel accelerated solvent extraction to achieve rapid extractions with minimal solvent requirements. In gas-assisted solvent extraction, hot extraction solvents and nitrogen gas are introduced into a stainless-steel cell, generating an extraction pressure of 200 psi (~14 bar). This combination of temperature and pressure significantly enhances extraction efficiency, reducing both time and solvent consumption compared to conventional techniques like Soxhlet.

In this application, the EXTREVA ASE Accelerated Solvent Extractor is used for the development of an analytical method to determine 15 PCDDs/PCDFs. Together with a GC-HRMS system, we developed an efficient and reliable sample preparation and analysis for these toxic compounds

Materials and methods

Sample preparation and extraction

To ensure a comprehensive and representative analysis, a sufficient sample size consisting of 30 soil samples was carefully selected. The soil samples were collected from various locations to encompass a range of environmental conditions and provide a comprehensive understanding of soil composition and contamination patterns. This substantial sample size allows for robust statistical analysis and enables reliable conclusions to be drawn regarding the characteristics, contaminants, and environmental implications associated with the studied soil samples.

Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N 22184-60101)
- Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS System
- Thermo Scientific™ Dionex™ 100 mL cell (P/N 22184-6221)
- Thermo Scientific™ Dionex™ Cellulose Filter (P/N 056780)
- Cape Technologies Dioxins and Furans Sample Preparation Materials[3]
- System Plus 250 mL Clear glass boston round (P/N GL250/SPC)
- System Plus 60 mL Clear glass EPA vial (P/N GV60/PC)
- Fisherbrand Disposable Culture Tube (P/N 14-959-35)
- Kimble 15 mL Disposable Centrifuge Tube Screw Thread Finish (P/N 73785-15)
- Canada Life Science 700 µL Amber Tapered bottom vial 8mm Crim (P/N 2261012)
- Thermo Scientific™ Crimp Top Seals 8mm (P/N C4008-1*)
- Phenomenex ZB-Dioxin Beta (60 m x 0.25mm x 0.20µm)

Solvents and chemicals

- Hexane (Fisher, P/N H303-4)
- Acetone (Fisher, P/N A929-4)
- Dichloromethane (Fisher, P/N D151-4)
- Toluene (Fisher, P/N T291-4)
- Methanol (Fisher, P/N A454-4)
- Isooctane (Fisher, P/N UN1262)
- Labelled DxF Stock Solution (Wellington Laboratories, P/N EPA-1613LCS)
- Internal Standard DxF Spiking Solution (Wellington Laboratories, P/N EPA-1613ISS)
- Thermo Scientific™ Dionex ASE™ Prep Diatomaceous Earth (DE) Dispersant, 1 kg (P/N 062819)

EXTREVA ASE Accelerated Solvent Extractor

Accelerated solvent extraction (pressurized fluid extraction)

Figure 1 EXTREVA ASE Accelerated Solvent Extractor

To ensure an effective and optimized extraction process, several key steps and considerations are implemented. Firstly, the preparation of the DxF working standard involves diluting a stock solution with methanol to achieve a final OCDD concentration of 2 ng/mL and 1 ng/mL for all the other compounds. Similarly, the DxF internal standard is 200 ng/mL.

The extraction procedure uses a 100 mL extraction cell body, tightly sealed with a hand-tightened cell cap. Careful insertion of a cellulose filter into the cell is performed using the dedicated tool. The process begins by mixing 10 g of dried soil sample with an appropriate amount of ASE Prep DE in a glass beaker. The resulting mixture is then transferred into the extraction cell, followed by the addition of 500 µL of the surrogate solution. Any remaining empty space in the cell is filled with ASE Prep DE to ensure proper packing. The second end cap is hand-tightened, and another cellulose filter is placed on top of the cell body.

To optimize the extraction process and facilitate efficient solvent contact, it is crucial to prevent sample compaction during the compression phase. Dionex ASE Prep DE (P/N 062819) dispersant plays a significant role in achieving proper sample dispersion, promoting effective solvent interaction. Additionally, effective removal of water from wet samples before extraction is essential. Two methods can be employed for this purpose. The first method involves freeze drying the samples, while the second method uses the proprietary Dionex ASE Prep Moisture Absorbing Polymer (P/N 083475). By mixing the samples with this polymer in a 1:1 ratio, optimal moisture removal is ensured, leading to improved overall performance during the extraction process. This approach also enhances recovery rates, contributing to the reliability and accuracy of the results. By incorporating these steps and employing the appropriate dispersant and moisture removal techniques, the extraction process can be optimized, resulting in efficient solvent contact and reliable analytical outcomes. The instrument was programmed with the parameters listed in Table 1 to create a hexane solution with a final volume of 170 mL, establishing ideal extraction conditions. Prior to sample extraction, the system underwent a thorough rinsing process using 30 mL of hexane. To further improve the purity and recovery of the concentrated solutions the samples is cleaned with sulfuric acid overnight. A two-stage acid silica-activated carbon coupled column system from CAPE Technologies was utilized. The initial step involved loading the hexane extracts onto the combined cartridge and subjecting them to a thorough hexane wash. The resulting wash solutions were collected and further processed using the column system 30 mL of hexane are collected through the acid silica. Subsequently, the carbon column was separated from the acid silica column, and a forward wash with 6 mL of 1:1 hexane: toluene was performed to capture the PCB fraction when combined.

Table 1. Accelerated solvent extraction (EXTREVA ASE Accelerated Solvent Extractor) settings

Extraction	
Cell Type	Stainless steel
Cell Size	100 mL
Oven Temperature	100 ℃
Purge Time	180 s (100 mL cell)
Nitrogen flow (gas assisted extraction)	10 mL/min per channel
Cell Fill Volume	70%
Solvent Flow Rate	5.0 mL/min
Extraction Solvent	Toluene-methanol 9:1
Extraction time	20 min
Extraction Volume	175 mL
Pre-run Rinse	30 mL, Hexane
Extraction Time (four samples)	42 min (100 mL cell, flow rate 5 mL/min)
Concentration	
Mode	Dryness
Collection flask	250 mL bottle
Rinse solvent (Extract)	Hexane
Evaporation temperature	70 ℃
Nitrogen flow rate	40 mL/min/sample
Vacuum	50 torr
Evaporation time (four samples)	120 min

Data analysis

This fraction was then ready for analysis. In contrast, the dioxin/furan fraction was obtained through reverse elution using 50 mL of toluene, followed by capture for analysis using magnetic sector GC-HRMS (Figure 2). Detailed conditions can be found in Table 2. The high-resolution capability of the system allows for the accurate identification and quantification of target compounds in complex samples. It provides detailed information about the elemental composition of the ions, enabling the differentiation of isobaric compounds and the detection of trace levels of contaminants. The magnetic sector analyzer offers superior mass resolving power, selectivity, and sensitivity, making it suitable for the analysis of highly complex mixtures in quantitative routine analysis.

Table 2. DFS magnetic sector GC-HRMS conditions

GC Conditions	
Injector	
Injector Type	Split/Splitless Injector
Liner	Restek Liner, Topaz, Single Taper, 4.0mm ID, 6.5 x 78.5mm, with Quartz Wool
Injector Temperature	220 ℃
Injection Mode	Splitless with Surge
Splitless Time	2.00 min
Split Flow	30 mL/min
Purge Flow	5 mL/min
Injected Volume	1 µL
GC	
Column	Phenomenex ZB-Dioxin Beta (60 m x 0.25mm x 0.20µm)
Carrier Gas	Helium
Flow Rate	1.3 mL/min, constant
Oven Temperature	80 ℃ (hold for 7.5min), ramp to 248 ℃ at 20 ℃/min, ramp to 252 ℃ at 0.2 ℃/min, ramp to 265 ℃ at 5 ℃/min, ramp to 285 ℃ at 3 ℃/min, ramp to 339 ℃ at 20°C/min (hold for 4.5min)
Mass Spectrometer Parameters	
Source Temperature	260 ℃
Ionization	EI
Electron Energy	50 eV
Transfer Line Temperature:	240 ℃
Acquisition Mode	MID

DFS MID Method (5 window)		DFS MID Method (5 window)	
Window 1	303.9016 305.8987 313.983 313.9834 lock 315.9419 317.9839 319.8965 321.8936 327.8847 331.9368 339.9339 341.8568 363.9802 cali 375.8364	Window 2	313.9834 lock 339.8597 341.8568 351.9 353.897 355.8546 357.8517 363.9802 367.8949 369.8919 409.7974 413.97 413.9770 cali
Window 3	363.9802 lock 373.8207 375.8178 383.8639 385.861 389.8156 391.8127 401.8559 403.853 413.977 413.9770 cali 445.7555	Window 4	407.7818 409.778 413.9770 lock 417.8253 419.822 423.7767 425.7737 435.8169 437.814 463.9738 cali 479.7195
Window 5	413.9770 lock 441.7428 443.7398 457.7371 459.7377 469.778 471.775 480.9691 501.97 501.9760 cali 513.6775		

Figure 2. DFS Magnetic Sector GC-HRMS System

Results

Accurate determination of isotopically labeled polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) is crucial in environmental and toxicological studies. The data reported in Figure 3 provide valuable insights into the average recovery percentages and relative standard deviations (RSDs) for isotopically labeled PCDDs and PCDFs, shedding light on the performance and reliability of the extraction method. The recovery rates obtained for the isotopically labeled PCDDs and PCDFs provide valuable information about the efficiency of the method in capturing and accurately quantifying the target compounds. Furthermore, the low (<20%) RSD values reflect the precision and reproducibility of the measurement process, bolstering the reliability of the results. Unlike other technologies, for high resolution magnetic sector field systems the LOQ and LOD values are based on the signal to noise concept. With the Thermo Scientific™ DFS™ TargetQuan Data Evaluation Software the user can define the parameters that form the basis of signal-to-noise calculation, such as the sigma value. The chromatogram visually presents the noise band to ensure complete transparency[4].

The data in Figure 4 represent the average concentrations and counts of various compounds obtained from a comprehensive analysis of 30 soil samples. These averages provide valuable insights into the typical levels and occurrences of the compounds within the sample set. Examining the average concentrations, certain compounds stand out due to their relatively higher levels. OCDD exhibits a particularly elevated average concentration of 809.51 pg/g, indicating its prominence in the soil samples. Similarly, 1234678-HpCDD and 1234678-HpCDF demonstrate substantial average concentrations of 151.43pg/g and 29.99pg/g, respectively. These findings suggest the potential significance and prevalence of these compounds within the soil environment. Analyzing the counts, it is apparent that the occurrences of each compound vary. OCDF has the highest count with 21 instances, indicating its frequent presence across the 30 soil samples. Conversely, 123789-HxCDF and 123789-HxCDD exhibit lower counts of 5 and 15, respectively, implying a relatively lower occurrence rate in the sampled soils. The combination of average concentrations and counts provides a comprehensive understanding of the data, enabling researchers to assess both the intensity and frequency of each compound's presence in the soil samples. This information plays a crucial role in environmental and toxicological studies, assisting in the identification of compounds that may warrant further investigation or require mitigation measures

Figure 3. Average recovery rates and RSDs for the isotopically labeled PCDDs and PCDFs.
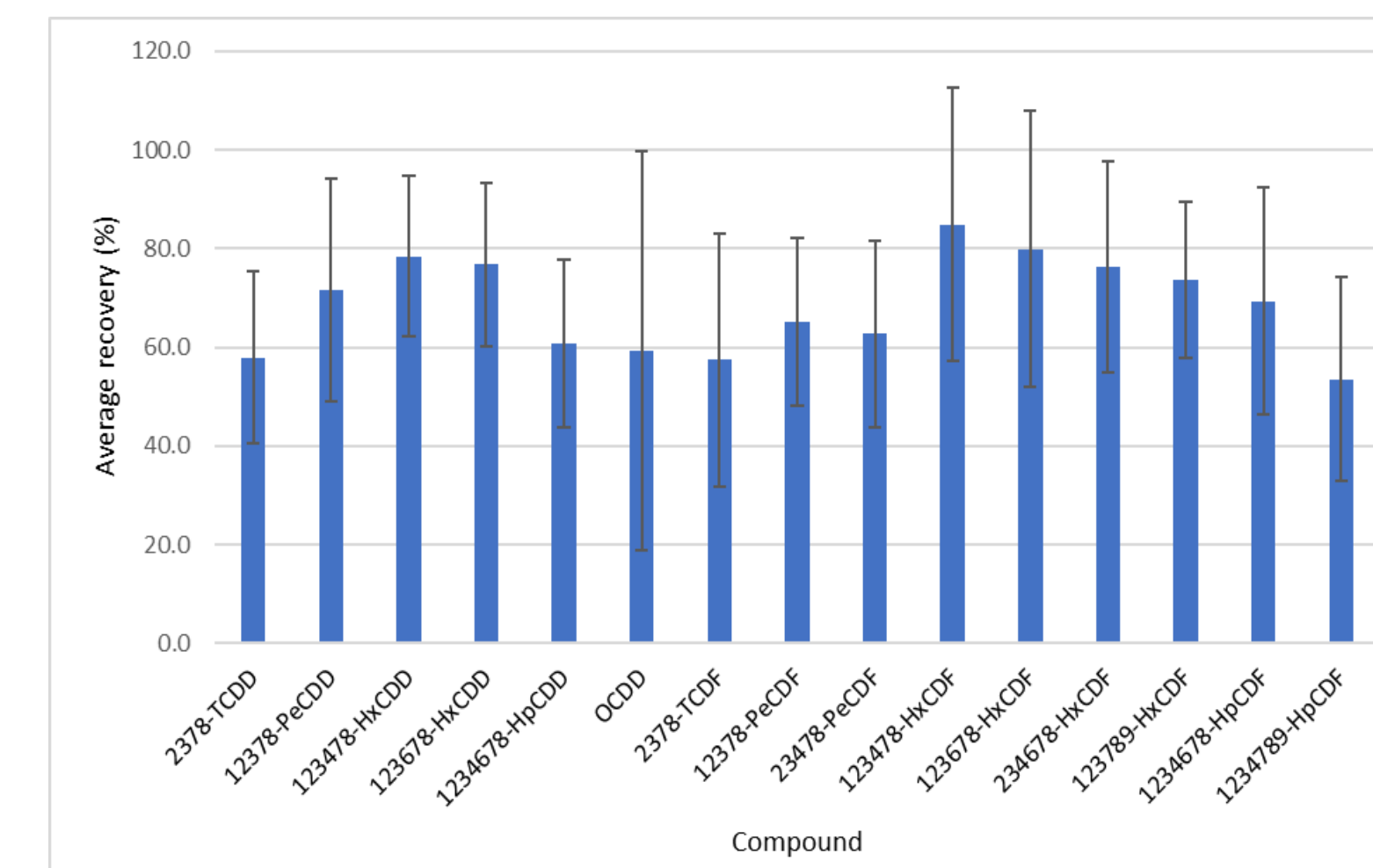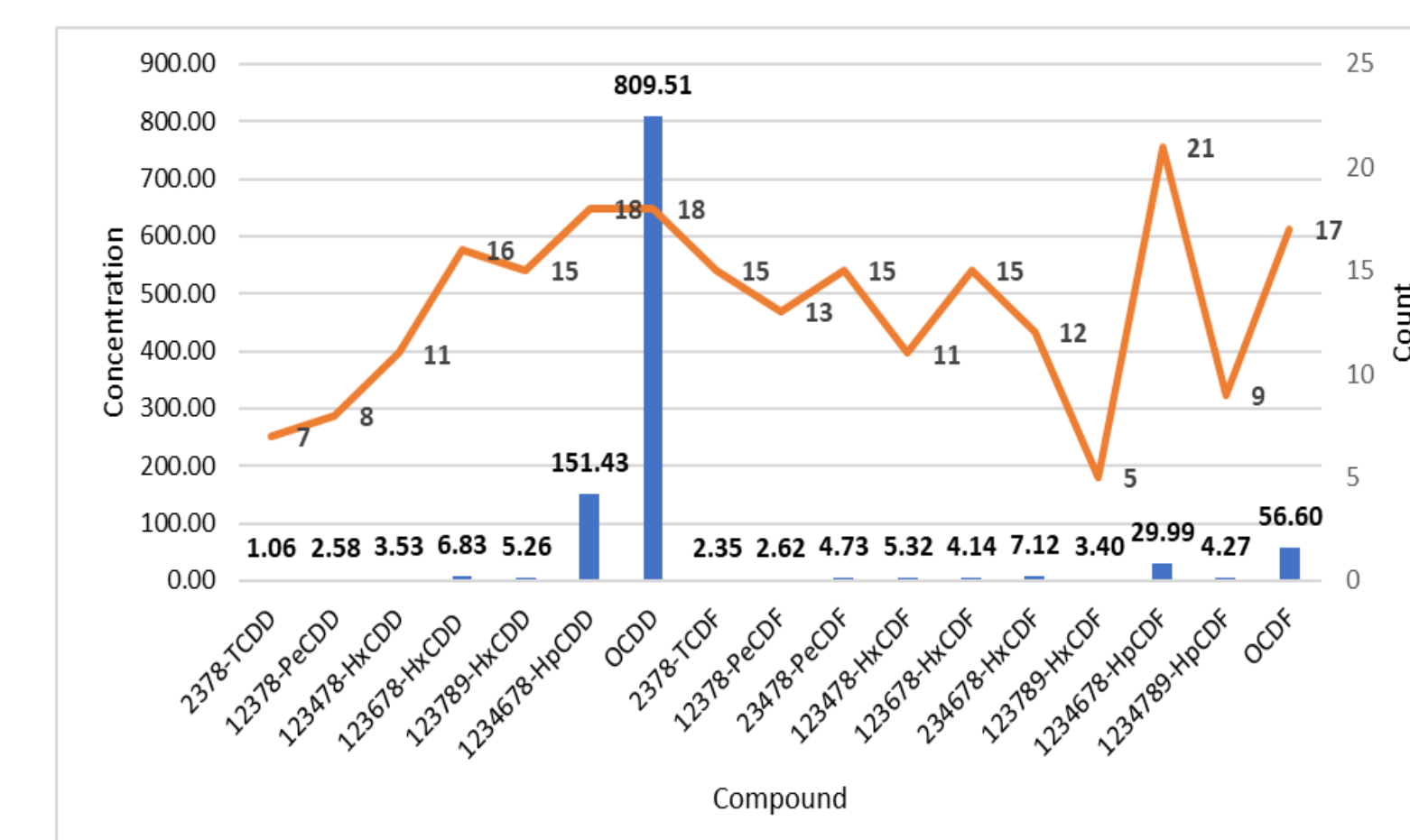

Figure 4. Average dioxin concentration in soil samples (n=30)

Conclusions

Accurate determination of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) is essential for conducting comprehensive environmental and toxicological studies. The provided data offers valuable insights into the average recovery rates, RSDs, concentrations, and counts of these compounds in soil samples, shedding light on their presence and behavior in the environment. The analysis of average recovery rates and RSDs demonstrate the effectiveness and reliability of the extraction method used. The high average recovery rates indicate the efficiency of the method in capturing and accurately quantifying the target compounds. Additionally, the low RSD values reflect the precision and reproducibility of the measurement process, enhancing the confidence and reliability of the results obtained.

Moreover, the examination of average concentrations and counts reveals important information about the occurrence and prominence of specific compounds in the sampled soil.

This combined information provides researchers with a comprehensive understanding of the data, allowing them to assess both the intensity and frequency of each compound's presence in the soil samples. Such insights play a crucial role in environmental and toxicological studies, assisting in the identification of compounds that may require further investigation or necessitate targeted mitigation measures.

References

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2. Kannan Srinivasan and Rahmat Ullah, Apparatus for parallel accelerated solvent extraction (U.S. Patent No. US 11,123,655 B2) Sep 21, 2021.

3. For more information about the preparation of samples for dioxin/furan analysis, please visit: http://www.cape-tech.com/, http://www.cape-tech.com/AN-008.pdf and http://www.cape-tech.com/TN-005.pdf

4. For more information about the Magnetic Sector GC-HRMS and its superior performance for quantitative routine analysis of dioxins and persistent organic pollutants (POPs) please visit: https://www.thermofisher.com/ch/en/home/industrial/mass-spectrometry/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-systems/high-resolution-gc-ms.html?gclid=CjwKCAjw44mIBhAQEiwAgP3eVq6FISoF609UGfmzX6Cq4qJGahtVmw5E8mVTCQzjN3OTxzXbmDUwJhoCplAQvD_BwE&ef_id=CjwKCAjw44mIBhAQEiwAgP3eVq6FISoF609UGfmzX6Cq4qJGahtVmw5E8mVTCQzjN3OTxzXbmDUwJhoCplAQAvD_BwE:G:s&s_kwcid=AL!3652!3!632581604355!!!g!!&gad=1#menu5

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