DETERMINATION OF SHORT POLYCHLORINATED ALKANES IN LEATHER USING GAS CHROMATOGRAPHY-ELECTRON CAPTURE NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

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ABSTRACT

Polychlorinated alkanes (PCA) are organic compounds of general formula $C_xH_{(2x+2-y)}Cl_y$. PCA are extremely complex industrial mixtures of randomly chlorinated petroleum distillate. They are made of several thousands structural isomers. The short chain isomers (sPCA) have a carbon number between 10 and 13. All PCAs have a high environmental persistence and accumulate in the biosphere. They were included in all regulatory programs implying reliable methods for their determination in various samples. The content of sPCA is regulated in leather. The objective of this study was to develop a reliable and sensitive analytical method to determine and to quantify sPCA in real-world leather samples. The protocol started by an ultrasound-assisted hexane extraction of the ground leather sample. The extract was purified by filtration on a Florisil® (magnesium silicate) column with hexane/dichloromethane 9/1 v/v eluant. The concentrated extract was redissolved in 5 mL isooctane for analysis with a gas chromatograph coupled with a mass spectrometer in the electron capture negative ionization mode. The method was validated and used with real-world samples giving a reliable limit of quantification of 2 mg/kg +/- 0.2 mg/kg for the sPCA family.

RESUMEN

Alcanos Policlorinados (PCA) son compuestos orgánicos de composición general $C_xH_{(2x+2-y)}Cl_y$. PCA son extremadamente complejas mezclas industriales de destilados del petróleo clorinados al azar. Son compuestos por varios miles de isómeros diferentemente estructurados. Los isómeros de cadenas cortas (sPCA) tienen entre 10 y 13 carbonos. Todos los sPCA tienen alta persistencia ecológica y se acumulan en la biósfera. Fueron incluidos en todos los programas regulatorios implicando métodos confiables para su determinación en varias muestras. El contenido de sPCA es reglamentado en el cuero. El objeto de este estudio fue desarrollar un método analítico para determinar y cuantificar con confiabilidad y precisión el contenido de sPCA en muestras de cuero obtenidas en un mundo real. La directiva empieza con la extracción con hexano auxiliada por medio de ultra-sonido sobre una muestra del cuero molido. El extracto se purificó por filtración a través de una columna con Florisil^{MR} (silicato de magnesio) con hexano/diclorometano 9/1 v/v cómo solvente de elución. El extracto concentrado se redisolvió en 5ml de isooctano para analizarse por medio de un cromatógrafo para gases acoplado a un espectrómetro de masa montado para capturar especies negativamente ionizadas. El método se validó con muestras ciertas para determinar un confiable límite de cuantificación de 2mg/kg +/- 0,2 mg/kg para productos de la familia de sPCA.

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INTRODUCTION

Polychlorinated alkanes (PCA), also known as chlorinated paraffins (CP), have the general formula $C_xH_{(2x+2-y)}Cl_y$. The number of carbon atoms, x, is between 10 and 30. The number of chlorine atoms depends on x usually being not less than 3 and more than 30. They are used in industrial applications since the early thirties as metal working fluids, plasticizers and flame retardants.[1] The PCA world production was around 10,000 metric tons in the 1950's steadily increasing 300,000 metric tons in 1995 to decrease slightly to 270,000 metric tons in 2005.[2] More than seven millions metric tons of PCAs have been produced since their introduction.[2] PCAs are very persistent in the environment explaining why they are found everywhere today. If they have a low acute toxicity, they also have a high potential for bioaccumulation. Bioaccumulation of some chlorinated chemicals, such as dichlorodiphenyltrichloroethane (DDT), was linked to delayed problems such as reduced fertility of birds and reptiles and/or cancers in animals that could be transferred to mankind.[3] DDT was definitively banned worldwide in 1972.[3] A significant toxicity for aquatic wildlife was established and caused the first restriction against PCAs to be taken by the Oslo-Paris (OSPAR) convention for the protection of the marine environment of the North-East Atlantic in 1998.[4] In 2004, the commercialization of industrial products containing more than 1% of PCAs was prohibited by the European directive 2002/45/CE.[5] This directive mentions specifically that all leather products should not contain more than 1% w/w of PCA used as fat liquoring. Later, in 2007, PCAs were listed in Appendix 17, of the European Registration, Evaluation, Authorization and restriction of Chemicals (REACH) regulation system as Substance of Very High Concern (SVHC).[6] PCA concentration in all finished products that can be in contact with the public must be lower than 0.1% in mass (1 g/kg or 1000 ppm).

If PCAs are regulated, their concentration in various matrices and samples must be controlled. PCAs are very complex mixtures of a huge large number of isomers. PCAs are sorted in three classes according to their carbon content: the short-chain PCAs, sPCA or short chain chlorinated paraffin (SCCP) class contains the C_{10}-C_{13} chloroalkanes, the medium-chain PCAs, mPCA or medium chain chlorinated paraffin (MCCP) class is made by the C_{14}-C_{17} polychlorinated isomers and the long-chain PCAs, lPCA or long chain chlorinated paraffins (LCCP) class covers the C_{20}-C_{30} more or less chlorinated waxes.

sPCAs seems to be the more toxic compounds of the polychlorinated alkane family.[1] Many works were published on sPCA determination particularly in environmental samples such as lake and river waters and sediments[2] or in biological tissue[4], but at our knowledge, no studies were performed specifically in leather samples. The techniques most often used to determine and quantify PCAs in environmental samples is gas chromatography (GC) coupled with electron capture detection (ECD) that is specifically sensitive to the chlorine content in the compound. Since the co-elution of other halogenated compounds is likely, a very selective sample clean up is required to avoid interferences. In some cases the clean-up was not sufficient and a second GC analysis with a different polarity column was necessary for full confirmation.[7] To obtain a signal independent of the chlorine content, the PCAs were catalytically dechlorinated on a metallic palladium wool placed in the hot injector, and the resulting alkanes were determined by GC using a classical flame ionization detector.[8] In most modern sPCA GC analyses, mass spectrometry (MS) is the most employed detection technique. The mass spectrometric mode most favorable with halogenated compounds is the electron capture negative ionization (ECNI) mode. The hyphenation with GC leads to the GC-ECNI-MS method.[9] Some methods were based on quantification with $[HCl]^{2-}$ and $[Cl_2]^-$ ions[10,11] and others with specific ions such as $[M-Cl]^-$[10, 12]. It was found that the MS signal was very dependent on the chlorine content of the PCA.[9-12] The electro-ionization tandem mass spectrometry (EI-MS/MS) mode was efficient. In this mode, fragment ions with low mass-to-charge ratios (m/z) coming from the original molecule are re-fragmented in the second MS mode. EI-MS/MS gave signals relatively less dependent on the chlorine content of the molecule and able to detect sPCA at concentration as low as one ng/g (ppb).[13] But the MS/MS capable equipment is expensive and not accessible in every laboratory. The GC-ECNI-MS alternative way was recently used associated to multiple linear regression analysis to determine independently the total carbon number and chlorine content of sPCAs. This work will focus on the sPCAs determination and quantitation in leather samples. A detailed approach of this class of molecules will be proposed, describing their physicochemical and toxicological properties. The GC-ECNI-MS method associated to multiple linear regression analysis will be applied to leather samples and optimized.

EXPERIMENTAL

Chemicals

Three standard solutions of technical sPCA C_{10}-C_{13} mixture with chlorine content of 51.5%, 55.5% and 63% were purchased from Dr. Erhenstorfer GmbH (Augsburg, Germany), each at a concentration of 100 mg/L in cyclohexane. Pure 1,1,1,3,10,11-hexachloro-undecane was selected as the internal standard for quantification. It was obtained from Chiron AS (Trondheim, Norway) as a 1 g/L (1000 ppm) solution in isooctane. Hexachloro-undecane, $C_{11}H_{18}C_{16}$, has a molecular weight of 363 and a chlorine content of 58.7%. Its polarity expressed as Log $P_{o/w}$ is 6.78. Isooctane of technical grade was supplied by VWR (Fontenay-Sous-Bois, France). Hexane and dichloromethane of Pestipur® quality were obtained from Carlo Erba-SDS (Val De Reuil, France). The magnesium silicate Florisil® with particle diameters between 150 and 250 µm (60-100 mesh) was purchased from Fluka (Sigma-Aldrich, St Louis, USA).

Sampling

The method was developed using a vegetable tanned goat leather sample that was cut in small pieces and then, mechanically ground to ~300 µm particles using a grinder (FRITSCH, Germany). For each test, 1 g of ground leather was weighted, put in a 20 mL flask and spiked with a known amount of a sPCA solution. Finally, 0.5 µg as 100 µL of the hexachloroundecane internal standard solution at 5 mg/L was added to the sample as 0.5 ppm in weight. The spiked samples were placed open in a hood for two hours to let all solvents evaporate at room temperature and sPCA and the internal standard diffuse evenly in the matrix before extraction. The method was applied to several real samples provided by our French customers. All tested leather samples were similarly ground and 1 g was put in a 20 mL flask with only 0.5 ppm (100 µL of 5 mg/L) of the internal standard added before the extraction step.

Extraction, clean-up

A Bioblock Scientific ultrasonic extractor 86482 (Elma, Singen, Germany) was used. 15 mL of hexane was added to each dried 1 g sample. The flasks were closed and placed in the ultrasonic bath. The temperature of water was set to 40°C and the extraction time was 1 h. The loaded hexane phase was filtered through a glass funnel filled with glass wool in a round bottom flask and reduced to about 1 mL in a R134V Rotavap® device (Büchi, Switzerland) with pressure and temperature set at 280 mbar and 40°C, respectively.

A 1 cm i.d. chromatography column was filled with a glass wool plug and 5 g of activated magnesium silicate Florisil® (length ~12 cm). Then, the prepared column was conditioned with 20 mL of dry hexane, and the concentrated extract was added at the column top. The column was first eluted with 10 mL of a hexane/dichloromethane (9/1, v/v) fraction and next by two 25 mL volume of the same solvent mix. The first 10 mL were discarded. The following 50 mL were collected. 1 mL of isooctane was added to the collected fraction that was concentrated to ~1 mL. Finally the concentrated eluate was transferred to a 5 mL volumetric flask and the volume was exactly adjusted to 5 mL with isooctane.

Chromatographic equipment

Quantification was performed on a gas chromatography GC-6890 (Agilent, Palo Alto, California) coupled with a mass spectrometer MSD 5975C (Agilent) equipped with a chemical ionization source.

A 2 µL sample was injected in pulsed splitless mode (0.35 MPa or 50 psi for 1 min), with an injector temperature set at 250°C. Helium (99,999%) was used as carrier gas at a constant flow of 1.2 mL/min. Separations were performed with a fused silica DB-5ms capillary column (15 m length, 0.25 mm i.d.) coated with a 0.25 µm thick film of phenyl arylene polymer (J&W scientific, Folsom, USA). The temperature program was as follows: after 2 min at 60°C for the splitless injection time (1 min) plus another min for equilibration, a 50°C/min ramp was started and lasted 4.4 min to reach 280°C. This temperature was maintained constant for 2.5 min, and a second 50°C/min ramp was programmed for 0.8 min to reach 320°C that was hold isothermal for 5.3 min. The total temperature program needs 15 min exactly. The transfer line between the column end and the MS source was set at 280°C. The ion source temperature was set at 230°C and the MS quadrupole temperature was set at 150°C. The mass spectrometer was operated in ECNI mode using methane (N45 99.995%, Air Liquide, France) as reagent gas with a 2 mL/min flow. Data were acquired in both single ion monitoring (SIM) and scan simultaneous modes and quantified with a multiple linear regression method described by S. Geiss et al. using the two specific ions with m/z 375 and m/z 423 to quantify sPCAs and m/z 364 for the internal standard.[14]

POLYCHLORINATED ALKANES PROPERTIES

Production

The major producers of PCAs in Europe are the British Imperial Chemical Industries (ICI) recently acquired by the Swedish company AkzoNobel, the French Atochem and the Italian Caffaro Chimica. In the US, the two chemical companies OxyChem (Los Angeles, CA) and Dover Chemical (Dover, OH) produce and sell more than half the PCAs needed by the local market. Numerous minor producers are present on the market and new comers are appearing in Asia compensating the declining PCA production by western countries and/or supplying increasing Asian local demands. The present world production of PCAs is estimated to have stabilized at about 270,000 metric tons/year.[2]

The PCA chemical synthesis is very simple. Three different petroleum distillate fractions (mostly mixtures of paraffins or *n*-alkanes): a C_{10}-C_{13}, a C_{14}-C_{17}, or a C_{20}-C_{28} fraction, are used. One of the three fractions is selected and melted heating between 80 and 100°C in a corrosion resistant reacting vessel. Chlorine gas is introduced in the vessel and the radical nucleophilic substitution reaction:

$$C_nH_{2n+2} + yCl_2 \dashrightarrow C_nH_{2n-y+2}Cl_y + yHCl \qquad (1)$$

takes place after initiation by UV light. The reaction propagates easily being exothermic with a reaction heat of about 150 kJ/mol Cl_2. A coolant system is needed to avoid overheating of the reacting vessel. Water is most commonly used to evacuate calories and to wash out of the waste gas all hydrogen chloride. Most plants work in batch processes adapting the reaction (paraffin weight and amount of chlorine added) to the customer demand.[15]

Commercial brand names are numerous. A non-exhaustive list includes the following trade names (producer in parenthesis): Adekacizer or ADK (Caffaro), Aquamix, Arubren (Atochem), Cereclor (ICI and Hoechst), Cloparin (Caffaro and Hoechst), Chlorcosane, Chlorez (Dover), Chloroflo, Chlorowax (Atochem), Clorafin, Creclor, Flexchlor (Witco/Argus), Hordalub (Hoechst and Leuna GmbH), Parachlor, Paroil clorez (Dover Chemical Corp.), Plastichlor, Toyoparax (Tosoh Corp.) or Unichlor (Union Carbide).

Physicochemical properties and composition

The alkane chlorination process substitutes randomly hydrogen atoms for chlorine atoms. This results in a massive number of compounds differing i) by the number of carbon atoms, ii) by the chlorine atom number and iii) by the relative position of the different chlorine atoms along the carbon chain. Since petrochemical refined paraffin fractions are used, they contain a majority of single linear *n*-alkanes associated with the numerous alkane homologues whose number increases exponentially with the alkyl chain length. For example, there is a single methane, ethane and propane alkane (C_1-C_3). There is also a single *n*-dodecane, but it is associated to 75 different dodecane isomers including the five methyl-undecanes, the sixteen dimethyl- and three ethyl-decanes, the trimethyl-, dimethyl-ethyl-, isopropyl- and propyl-nonanes and so on. The single *n*-hexadecane is associated to 476 different structural isomers; the *n*-eicosane (C_{20}) has 4762 structural isomers and the triacontane (C_{30}) 189,584.[16]

All these alkane isomers are randomly substituted with chlorine atoms producing a huge number of different isomers in any PCA sample. For example, the internal standard selected was 1,1,1,3,10,11-hexachloro-undecane. There are 516 different isomers of linear *n*-hexachloro-undecanes. If we consider that there are 48 different structural isomers of undecanes that could be randomly substituted by six chlorine atoms, the product 48x516 gives an estimated number of 24,768 structurally different hexachloro-undecane.[16] The number is so large that even to most efficient GC columns is unable to separate them individually. Clumps of peaks are always obtained in PCA GC analyses. The numbers of structural isomers only are mentioned. Chiral stereoisomers due to the all sp^3 carbon hybridization of chloroalkanes could multiply the isomer numbers by an order of magnitude.[16]

Considering the number of possible isomers and the PCA production process, the chlorine content of a PCA sample is considered. The chlorine content is expressed in percentage of the chlorine mass over the molecule mass. Table 1 lists the chlorine content of the sPCA isomers. Figure 1 is a graphic representation of the PCA chlorine content for the three classes: sPCA (in green), mPCA (in pink) and lPCA in dark blue. The inset is an enlargement of the sPCA curves since this class is the studied one. Figure 1 clearly shows that chlorine content and carbon chain length are independent. For example, all three PCA classes can contain 50% chlorine. A 50% chlorine content in a sPCA sample means that the PCAs contains an average of four to five chlorine atoms per molecules. The molecules of the mPCA sample with 50% chlorine content have between six and seven chlorine atoms each and those of the lPCA sample have an average of eight to twelve chlorine atoms per molecule. Obviously if the chain length increases, similar chlorine content will require more chlorine substitution. Considering the large number of possible isomers, the 50% chlorine sPCA sample contain a major part of four or five chlorine substituted molecules, it also contains molecules with only one, two or three chlorine atoms. These "under" chlorinated molecules are compensated by a similar number of "over" chlorinated ones with six, seven or eight chlorine atoms per molecule, likely forming a Gaussian curve.

The physicochemical properties of the PCA obviously depend on the carbon chain length and the chlorine content. The PCA vapor pressures are very low at room temperature, the highest being 0.03 Pa for sPCAs with low chlorine content (40 %) and the lowest being an estimated 10^{-23} Pa for lPCAs with high chlorine content (70%).[17] The PCA viscosities increase with the carbon content and even more with the chlorine substitution (Table 2).

Hydrophobicity

The water solubility decreases rapidly with the carbon chain length and increases somewhat with the number of chlorine atoms in the molecule. The accepted parameter to estimate molecule hydrophobicity in pharmaceutical, environmental and biological studies is $P_{o/w}$, the octanol/water partition coefficient, expressed as its log value. Table 2 lists the average water solubilities and log $P_{o/w}$ value for the three classes of PCAs.

Uses

The major uses of PCAs are additives in a wide variety of products: they are used as plasticizers in PVC, rubber, sealants, paints and varnishes. They are commonly used as flame retardant in e.g. textiles, plastics and tires. This flame retardant property is associated with the PCA ability to repel water and prevent molding in leather products and textiles. sPCAs were commonly used as liquoring agent in leathers maintaining flexibility and softness and decreasing aging associated to brittleness. A significant mass proportion of PCAs is used as additives in high temperature lubricants for metal working and/or cutting oils.

RESULTS AND DISCUSSION

Knowing the huge number of PCA isomers in any sample, it is certain that it will not be possible to separate each isomer individually. It is required to work with PCA as a class of molecules. The case of the determination of short chain PCAs in leather sample is fully described.

TABLE 1:
Chlorine content of individual short chain (C_{10}-C_{13}) polychlorinated alkanes (sPCA).

Carbon	Hydrogen	Chlorine	Mass Cl	m.w.	chlorine content w%
10	21	1	35.5	176.5	20.1%
10	18	4	142	280	50.7%
10	16	6	213	349	61.0%
10	14	8	284	418	67.9%
10	7	15	532.5	659.5	80.7%
10	0	22	781	901	86.7%
11	23	1	35.5	190.5	18.6%
11	22	2	71	225	31.6%
11	19	5	177.5	328.5	54.0%
11	17	7	248.5	397.5	62.5%
11	15	9	319.5	466.5	68.5%
11	9	15	532.5	673.5	79.1%
11	0	24	852	984	86.6%
12	25	1	35.5	204.5	17.4%
12	23	3	106.5	273.5	38.9%
12	22	4	142	308	46.1%
12	20	6	213	377	56.5%
12	18	8	284	446	63.7%
12	12	14	497	653	76.1%
12	5	21	745.5	894.5	83.3%
12	0	26	923	1067	86.5%
13	27	1	35.5	218.5	16.2%
13	26	2	71	253	28.1%
13	24	4	142	322	44.1%
13	21	7	248.5	425.5	58.4%
13	19	9	319.5	494.5	64.6%
13	17	11	390.5	563.5	69.3%
13	9	19	674.5	839.5	80.3%
13	3	25	887.5	1046.5	84.8%
13	0	28	994	1150	86.4%

Optimisation of extraction and clean-up procedure

The standard procedure for that analysis of anything in a leather sample starts with a reproducible grinding of the sample. Next, ultrasound-assisted solvent extraction is often a good choice to extract the leather sample because ultrasounds make transfer of analytes to solvent easier facilitating the solvent penetration in leather pores. Hexane was used as solvent because the solubility of sPCAs is good in hexane and few undesired polar compounds are co-extracted. After optimisation of the temperature, time of extraction and number of extraction cycles, it was established that all leather samples were best extracted with two cycles of 1 hour at 40°C, each done using 15 mL of hexane per gram of ground leather. Leather is a complex matrix that contains many apolar compounds such as fat or chemical agents used for tanning process that could be co-extracted with PCAs. So a special clean-up procedure must be optimized to insure that a reliable PCA determination will be possible. Given the PCA number of isomers, it is important to be sure that the clump of chromatographic peaks corresponding to PCA isomers (Figure 2) is not overlapped by any other alien apolar compounds.

TABLE 2: Physicochemical properties of the three PCA classes.

PCA class	Chain length	Chlorine content	state	Viscosity mPa.s	Vapor pressure Pa	Water solubility µg/L or ppb	log $P_{o/w}$	Toxicity
Short chain	C_{10}-C_{13}	43%	Liquid	50	0.001-0.1	0.9 – 1.26	5 – 6	Very toxic to aquatic organisms, carcinogenic
		50%	Liquid	~1000	10^{-6}-0.001	0.45 – 0.90	6 – 8	
Medium chain	C_{14}-C_{17}	40%	Liquid	~100	10^{-4}-0.02	0.5 – 1.1	6 – 7	Toxic to aquatic organisms, potentially carcinogenic
		46%	Liquid	~300	10^{-7}-10^{-4}	0.05 – 0.8	7 – 8	
		52%	Syrup	~3000	10^{-9}-10^{-12}	0.029 – 0.1	8 – 9	
Long chain	C_{20}-C_{30}	35%	Liquid	~100	10^{-8}-10^{-5}	0.001– 0.0086	9 – 10	No clearly established carcinogenic properties
		44%	Syrup	~1000	10^{-12}-10^{-9}	10^{-4} – 0.001	10 – 11	
		49%	Wax	~5000	10^{-17}-10^{-13}	$1.6x10^{-6}$ – 10^{-4}	11 – 13	

Data from Refs 1, 7, 9 and 17 given at room temperature (20-25°C). Vapor pressure estimated using the relationship: log (vapour pressure) = -0.353 nC – 0.645 nCl + 4.462 from Ref. 17. The viscosity unit 1 mPa.s corresponds to the centipoise (cP).

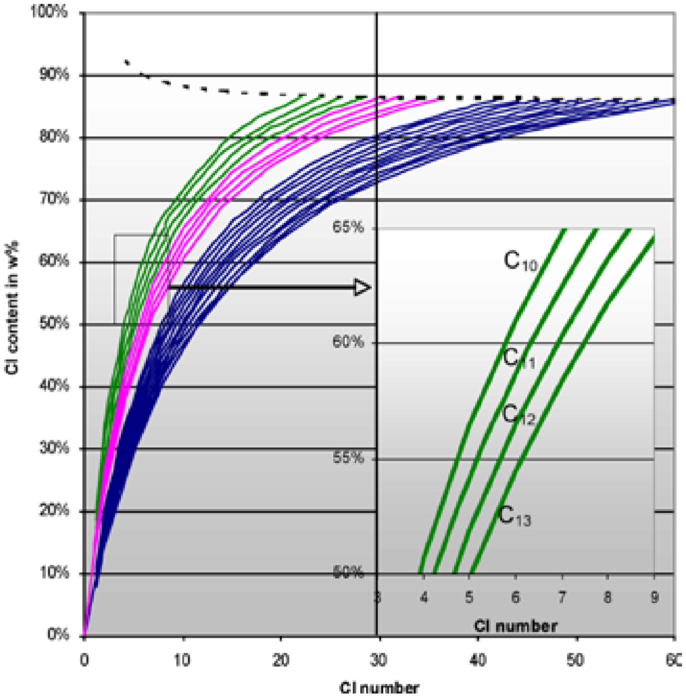

Figure 1: Relationship between the average number of chlorine atom per PCA molecule and the experimental chlorine content. Green lines: short chain C_{10}-C_{13} PCAs; pink lines: medium chain C_{14}-C_{17} PCAs; and dark blue: long chain C_{20}-C_{30} PCAs. The top dashed line corresponds to the maximum possible chlorine content for the C_xCl_{2x+2} fully halogenated (perchloro) alkanes. The inset figure is an enlargement of the sPCAs corresponding to the samples tested in this study.

Chromatographic filtration on Florisil®, an activated magnesium silicate derivative, was an efficient way to eliminate all fat. This filtration is the most popular method to phase apolar compounds out of the leather hexane extract.[11, 18] It was first though that the PCA extract was correctly cleaned using two portion of 30 mL pure heptane on a 5 g, 12 cm Florisil column. However, the purification of hexachloro-undecane spiked samples demonstrated that the recovery was less than 80%. Adding 10% dichloromethane to the eluant to increase its polarity allowed for full recovery of the spiked sample. But some fatty compounds appeared overlapping with PCAs in the GC chromatogram. Performing the elution with three portions of hexane/dichloromethane 9/1 v/v, the first portion of 10 mL was discarded containing the fat (triglyceride) part of the hexane extract. The two following 25 mL portions gave good results with a 99% recovery of the spiked PCA sample.

PCA quantification

The method was designed to quantify sPCAs with, by definition, carbon chain length between 10 and 13 and average chlorine content between 49 and 67% (between 2 and 11 chlorine atoms per molecule).[14] The response factors of the ECNI-MS detector is very depending on the chlorine content. To overcome this problem, a calibration using synthetic mixtures containing different chlorine content is performed integrating two different *m/z* values over the full retention range of the sample. The integrated values for the two *m/z* values are related to the synthetic mixture known concentrations by multiple linear regression calibration.[14] The selected *m/z* peaks must minimize

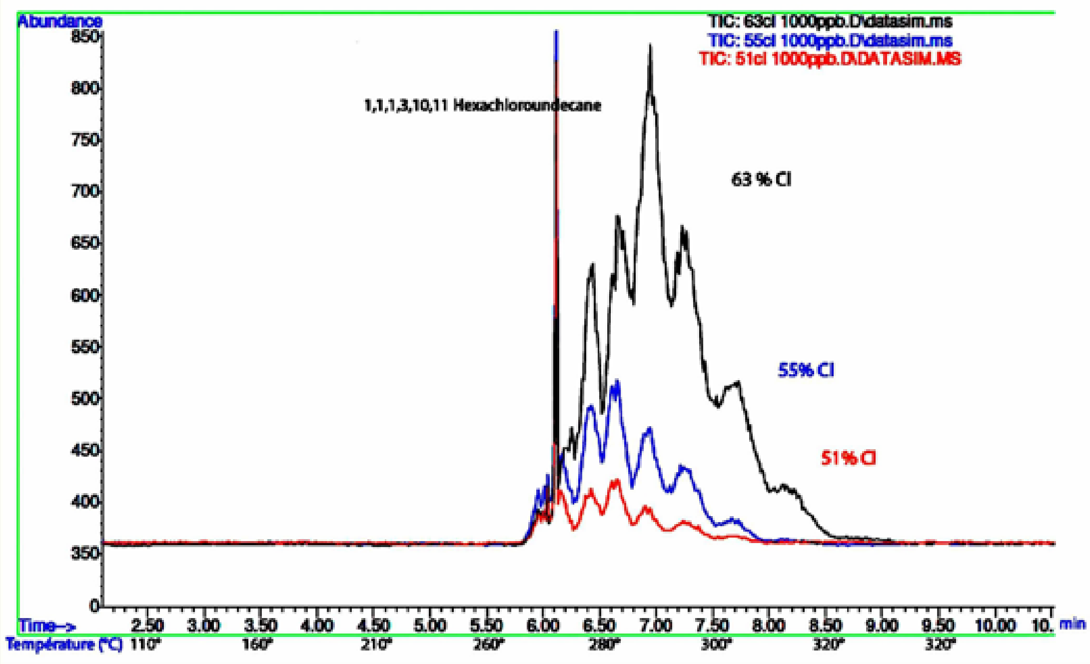

Figure 2: Overlay chromatograms of sPCAs with 51.5%, 55.5% and 61% chlorine content (massif of peaks between 5.8 and 9.0 min) and hexachloro-undecane (fine peak at 6.10 min) in SIM mode (ion MS current for the sum of m/z 327, 375, 409, 423, 362 and 364). The abscissa has both a time (min) scale and the corresponding column GC temperature scale.

all potential interferences. The original article recommended *m/z* 327 (ions with 11 C and 5 Cl, Table 1) and *m/z* 423 (ions with 13 C and 7 Cl) working with aqueous samples and sediments.[14] After several experiments, the two selected *m/z* peaks were 375 (ions with 12 C and 6 Cl) and 423.

Calibration was achieved with three sPCA solutions with chlorine content 51.5% (an average of four to five chlorine atom per molecule), 55.5% (four to six Cl/molecule) and 63% (five to eight Cl/molecule, Figure 1 inset). The selected concentration range was between 0.15 mg/L and 1 mg/L of synthetic sPCA with 0.1 mg/L hexachloro-undecane added as internal standard. Table 3 lists the compositions prepared to obtain enough data to perform a significant regression calibration.

The calibration procedure was validated by a MAV approach (maximum acceptable variation) as described in the AFNOR protocol NF T 90 210 with five inter-day tests.[19] Concentration levels were recalculated by multiple linear regressions following the Geiss method.[14] The recalculated concentrations must be within 45% or less of the actual experimental concentration values. Figure 3 shows the exact line (slope 1) with no error between the calculated and actual concentrations. The upper and lower dotted lines corresponds to the maximum (+45%) and minimum (-45%) tolerated calculated values. All our Table 3 samples gave acceptable calculated concentrations working with the two *m/z* peaks at 375 and 423 (Figure 3). The LOQ was validated by spiking two leather samples at 2 mg/kg during five days as described in the AFNOR protocol NF T 90 210. The calculated average concentration with the incertitude (more or less two calculated standard deviation) must be between the LOQ +/- 45% as represented in figure 4.

Real Samples

In 2010, CTC laboratory received 94 samples of leather or textile for sPCA analysis. The presented method was used for all 94 sPCA determinations. 89 samples were negative showing no detectable trace of sPCA. Only five samples gave a positive result. In these five sPCA containing samples, two contained just traces below the method limit of quantification (2 mg/kg). The three remaining samples contained quantifiable sPCA amounts. Full results and information about these five positive samples are given in Table 4. The highest containing sample with a quantified 220 mg/kg sPCA content is still acceptable in the European Union being five time below the tolerated 1000 mg/kg (0.1% w/w) limit.

CONCLUSION

This work presents a relatively simple analytical method to determine and to quantify short chain PCAs in leather samples. This PCA quantification was not previously possible in our laboratory where sPCAs were qualitatively determined by capillary GC with electron capture detection. The proposed procedure starts with an ultrasound-assisted hexane extraction of the ground leather sample. The hexane extract is cleaned-up by a Florisil® column filtration that will remove all fatty

TABLE 3:
Compositions of the calibration solutions for sPCAs with chlorination level between 51.5% and 63%.

Calibration solution	Concentration of standard solutions in mg/L			
	Internal Standard	sPCA 51.5 %	sPCA 55.5 %	sPCA 63 %
1	0.1	0.15		
2	0.1		0.15	
3	0.1			0.15
4	0.1	0.6		
5	0.1		0.6	
6	0.1			0.6
7	0.1	1		
8	0.1		1	
9	0.1			1

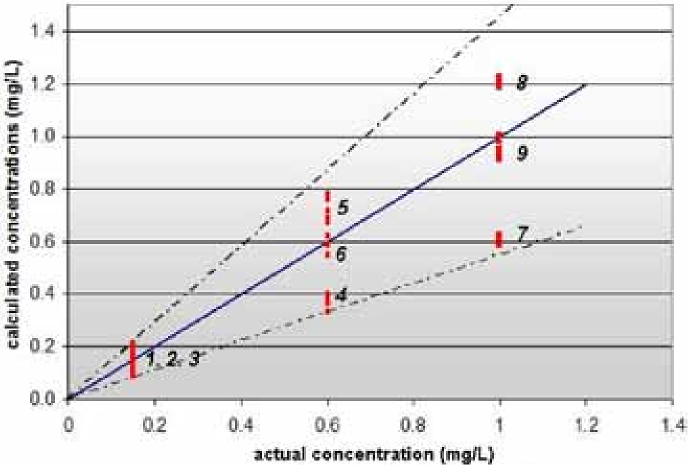

Figure 3: Calibration validation of the Table 3 synthetic mixtures by maximum acceptable variation (MAV) approach. The numbers refer to Table 3 calibration solutions.

components extracted. The sPCA content in the purified hexane solution is determined associating capillary GC with electron capture negative ionization mass spectrometry (ECNI-MS). A multiple linear regression procedure with a set of nine calibrated sPCA solutions allowed for a reliable quantitation acceptably independent of the PCA chlorine content down to a limit of quantification of 2 mg/kg of sPCA in the leather sample corresponding to 400 ng/mL for the solution injected in the GC. The method was validated for short chain PCAs with a chlorination level ranging between 51.5% to 63%. It was then successfully used to control the sPCA content in all received leather samples from our customers.

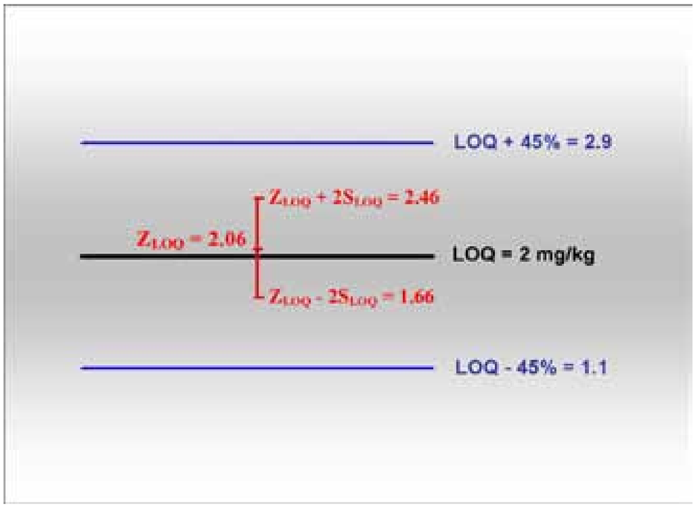

Figure 4: Validation of LOQ at 2 mg/kg in leather corresponding to an extracted concentration of 400 ng/mL injected in the GC apparatus. Z_{LOQ} is the computed average concentration in solid leather (ten measurements) and S_{LOQ} the corresponding calculated standard deviation giving two experimental limits well within the theoretical +/- 45% limits.

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TABLE 4:
Information about samples tested positive to sPCA

Product	Origin		Tanning	Appearance / Finishing	Sample use	sPCA concentration (mg/kg)
	Geographic	Animal				
leather	EU	calf	chromium	blue nubuck probably oilskined	shoes	5.4
leather	EU	calf	chromium	brown corrected-grain pigmented	shoes	<LOQ
leather	EU	calf	chromium	White full-grain pigmented	shoes	3.6
leather	EU	(calf)	chromium	split leather, black suede	shoes	<LOQ
coated textile	unknown	/	/	black polymer coat / green textile	Bag for boots	220

EU: European Union; LOQ: limit of quantitation (2 mg/kg)

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