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Effect of home cooking processes on chlordecone content in beef and investigation of its by-products and metabolites by HPLC-HRMS/MS



Deborah Martin, Fiona Lobo, Gwenaëlle Lavison-Bompard, Thierry Guérin, Julien Parinet*

Université de Paris-Est, ANSES, Laboratory for Food Safety, 94700 Maisons-Alfort, France

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ABSTRACT

Chlordecone (CLD) is a toxic organochlorine pesticide frequently used in the French West Indies until 1993, resulting in a contamination of soil and food. This study assessed the behaviour of CLD residues and CLD processing factors (PFs) during four home cooking processes: cooking in a conventional oven (“oven”), frying (“pan”), cooking in a microwave oven (“microwave”) and grilling (“grill”). These four processes were applied to six types of naturally contaminated beef (kidney, liver, rib, chuck, top-sirloin and sirloin). Targeted analyses with isotopic dilution were carried out by ID-HPLC-MS/MS to determine CLD concentrations before and after each cooking process and the corresponding processing factors. HPLC-HRMS/MS was used to find potential organochlorine degradation by-products and/or CLD metabolites present in samples by target, suspect and non-target screening. Cooking processes and especially microwave cooking led to a significant decrease in the CLD contained in beef ($2\% < PF < 17\%$). Traces of 5b-hydro-CLD and of another mono-hydro-CLD were found in the uncooked liver but no CLD degradation by-product was observed in the cooked liver.

1. Introduction

Chlordecone (CLD) is an organochlorine pesticide frequently used from 1972 to 1993 in the French West Indies to fight the black banana weevil (*Cosmopolites sordidus*) (Le Déaut and Procaccia, 2009). CLD was banned in France in 1990 but continued to be used until 1993. CLD is very stable, poorly soluble in water, and remains in the soil and vegetables for years with a low release rate (Cabidoche et al., 2009). This highly-toxic compound (Muckle et al., 2013; Multigner et al., 2010) has been registered since 2009 as a persistent organic pollutant (POP) on the Stockholm Convention list. A causal relationship between CLD exposure and an increased risk of prostate cancer in humans has been demonstrated (Guldner et al., 2010). In Martinique and Guadeloupe (Le Déaut and Procaccia, 2009), more than 8% of the usable agricultural area are highly polluted (CLD concentration greater than 1 mg/kg soil dw) and farm animals may be contaminated by fodder, water or involuntary soil ingestion. They may in turn contaminate humans (Fournier et al., 2017). Indeed, nowadays, due to their diet, 92.5% of the Martinican people and 94.9% of the Guadeloupean people have CLD concentrations above the detection limit in their blood serum (Dereumeaux et al., 2019). Unlike other lipophilic POPs, fat is not the target tissue for CLD, which is mainly stored in the liver (Guzelian, 1982; Lastel et al., 2016; Rychen et al., 2013; Soine et al., 1983) but can

also be found in muscle tissues. Dedicated CLD surveillance plans have been implemented in Guadeloupe for several years. In 2017, eight samples out of 327 bovines analysed (Ministère de l'Agriculture et de l'Alimentation & Ministère de l'Economie et des Finances, 2018), exceeded the previous maximum residue limit (MRL) set by the European Union at 100 ng CLD.g⁻¹ in fat for bovines. In 2019, the MRL was revised by the French government, and now stands at 20 ng CLD.g⁻¹ of fresh meat for bovines in muscle. Nevertheless, the black market (not covered by surveillance plans) is the main CLD contamination route for the population (Anses, 2017).

In 2014, Clostre et al. studied the effect of home food processing with a maximum temperature of 100 °C, on the CLD content in vegetables. Among their conclusions, they highlighted the fact that boiling vegetables in water had no effect on CLD content and consequently on consumer exposure. However, as far as we know, no study has been carried out with temperatures higher than 100 °C. Indeed, meat is usually cooked at higher temperatures (between 100 °C and 220 °C), and for different times (5 min–2 h), depending on the various cooking processes involved and type of matrix eaten. Moreover, many cooking-process studies have been conducted on chlorinated pesticides in meat at temperatures above 100 °C, but none on CLD. For example, Sengupta et al. (2010) assessed the impact of different cooking processes on chlorinated pesticides in meat. Their results indicated that food

* Corresponding author.

E-mail address: julien.parinet@anses.fr (J. Parinet).

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processes (cooking and baking) induce a decomposition of all the studied pesticides (HCH, DDT, endosulphan, malathion and dimethoate). Bayarri et al. (1994) studied the fate of pp'DDT in ovine meat after home cooking processes (roast, grilled and cooked meat) with temperatures ranging from 85 °C to 130 °C. A decrease in pp'DDT concentration of around 80% was observed. These results are corroborated by the study of Duvall and Rubey, (1976), whose thermogravimetric analysis suggested the simple evaporation of DDT between 100 °C and 170 °C. A similar thermogravimetric experiment was conducted by the same authors on CLD and they concluded that CLD evaporated at temperatures ranging from approximately 95–195 °C. To evaluate the fate of pesticides having undergone food processing, the OECD (OCDE/OECD, 2008) has recommended using the processing factor (PF), estimated by the ratio of residue level in processed commodities to that in raw commodities (Han et al., 2016; OCDE/OECD, 2008). No results are currently available on CLD's PF values for meat at temperatures corresponding to those commonly used to cook it. Therefore, the determination of CLD's PFs for various cooking processes is essential to more accurately evaluate human exposure to this molecule as mentioned in the OECD guidelines (OCDE/OECD, 2008). During cooking processes, chlorinated compounds can be converted to potentially toxic degradation by-products (Kubacki and Lipowska, 1980). In the study mentioned above, Bayarri et al. (1994) reported that DDT is converted to DDD and DDE after cooking processes. No information is yet available on the degradation of CLD during home cooking processes.

In the 1970s, gas chromatography combined with quadrupole mass spectrometry (GC-MS/MS) was the standard method for the analysis of CLD (Harless et al., 1978). Over the years, the combination of liquid chromatography with mass spectrometry (HPLC-MS) has gradually replaced GC-MS/MS due to its increased sensitivity (Saint-Hilaire et al., 2018). Recently, Saint-Hilaire et al. (2018) published a method using QuEChERS-HPLC-MS/MS in multi-reaction method (MRM) mode to analyse CLD in ewe livers using isotopic dilution (IS), and a limit of quantification (LOQ) of 1.36 µg CLD kg⁻¹ of fresh meat was validated. With the HPLC-MS/MS MRM mode analysis, however only pre-selected compounds can be found and any others are missed. Cooking processes may convert pesticides to by-products, which could be potentially toxic as mentioned before (Bayarri et al., 1994). They therefore need to be identified. Accordingly, a high-resolution mass spectrometer (HRMS) combined with HPLC seems to be appropriate for overcoming the deficiencies of the previously mentioned techniques. HPLC-HRMS does not require standards and can be used without *a priori*. The higher resolution of these instruments makes it possible to obtain the exact mass of each compound and to implement target, suspect and non-target screening (Bade et al., 2019; Belghit et al., 2015; Kunzelmann et al., 2018; Léon et al., 2019; Masiá et al., 2016).

The aim of this research was to measure the distribution of CLD in a naturally contaminated bovine through its meat, and to evaluate the effect of several home cooking processes with temperatures higher than 100 °C on the CLD content of this beef. CLD residues and PFs were assessed by isotopic dilution and HPLC-MS/MS analysis. Particular attention was paid to the detection of potential organochlorine CLD by-products and metabolites in bovine livers before and after various home cooking processes using an HPLC-HRMS/MS instrument with target, suspect and non-target approaches. Finally, two annotation algorithms were compared in order to evaluate their performance and efficiency in delivering the right raw formula for real contaminated samples.

2. Materials and methods

2.1. Chemical substances

The main physico-chemical properties of CLD are presented in Table SD-1. Chlordecone and ¹³C₈-chlordecone (purity > 98%) were both supplied by Azur Isotopes SAS (Marseille, France) and 5b-hydrochlordecone (5b-hydroCLD) (unknown purity and concentration) was

supplied by CEA-Genopole (Evry, France). The isotope-labelled CLD standard (¹³C₈-chlordecone) supplied by Azur Isotopes SAS (Marseille, France) was used as an internal standard (IS). Working solutions were prepared in HPLC-MS-grade acetonitrile obtained from VWR (Radnor, United States) for CLD and its corresponding IS. The solutions were stored at 4 °C in amber vials until use. Acetonitrile, obtained from Fisher Scientific (Illkrich, France), and ultrapure water (18.2 MΩ.cm), obtained by purifying distilled water with a Milli-Q system from Merck Millipore (Saint-Quentin-en-Yvelines, France), were used for the mobile phases and sample preparation. Formic acid (purity 98%), used for preparing the mobile phases, was supplied by Fisher Scientific (Illkrich, France). QuEChERS extraction kits for the EN 15662 method were provided by Agilent Technologies (Santa Clara, USA): each kit contained 4.0 g of magnesium sulphate (MgSO₄), 1.0 g of sodium chloride (NaCl), 1.0 g of sodium citrate, and 0.5 g of sodium citrate sesquihydrate. Supel QuE PSA/C18 [150 mg of polymerically bonded, ethylenediamine-N-propyl phase that contains both primary and secondary amines (PSA), 150 mg of C18, and 900 mg of MgSO₄] was obtained from Agilent Technologies.

2.2. Equipment and apparatus

A Proline Cooky oven (Darty, Bondy, France) and a Pyrex dish (Corning, USA) were used jointly for the "oven" processes. A Bluesky temperature-controlled induction hob (Carrefour, Boulogne-Billancourt, France) and a Tefal-coated frying pan (17 cm in diameter) (SEB, Rumilly, France) were used for the "pan" processes. A Family Chef combination microwave oven (Whirlpool, Puteaux, France) was employed for the "microwave" processes and grilling ("grill" mode).

An Eppendorf centrifuge 5810 (Hamburg, Germany) and a Genie 2 vortex mixer (Scientific Industries, Bohemia, USA) were used for the extraction procedure. An IKA Ultra-turrax T25 digital homogeniser and a GM 200 Grindomix (Retsch Technology GmbH, Haan, Germany) were used for the extraction procedure. A Mettler Toledo (Columbus, USA) XS 204 analytical balance was used to weigh the samples.

An Agilent 1200 series binary pump and autosampler (Santa Clara, USA) coupled with a 5500 Q-Trap AB SciEX mass spectrometer (Darmstadt, Germany) with an electrospray ionisation source (ESI) was used for the quantitative experiments.

An HPLC Thermo Fisher Scientific U3000 system composed of a binary pump and autosampler (Waltham, USA) coupled with a 5600 Q-TOF AB SciEX mass spectrometer (Darmstadt, Germany) with an electrospray ionisation source (ESI) was used for the qualitative experiments.

For both LC-MS techniques, a Phenomenex Aqua C18 column (150 mm × 2.0 mm × 3.0 µm) and an Aqua C18 precolumn (4.0 mm × 2.0 mm × 3 µm) were used for chromatographic separation.

2.3. Sample preparation

The beef samples used in the present research came from an animal naturally contaminated by CLD, found positive in 2018 by the French health services in Martinique (French West Indies) with a level over the MRL (set at that time at 100 ng CLD.g⁻¹ in fat) and not fit for consumption. Six types of meat commonly consumed by inhabitants were investigated: chuck, top-sirloin, sirloin, rib, kidney and liver.

In order to ensure the homogeneity of experiments, the following sampling procedure was used. Firstly, 250 g of each piece of meat was sampled and cut into 25 equal parts. Secondly, the 25 parts were randomly mixed to form five groups: one for measuring the uncooked concentration of CLD and four for measuring the impact on CLD of each of the four home cooking processes implemented in this study, i.e. frying ("pan"), cooking in a conventional oven ("oven") or a microwave oven ("microwave") and grilling ("grill").

Around 50 g of meat was cooked by each cooking process. The meat

Table 1
Performance of the QuEChERS-HPLC-MS/MS analytical method used to measure CLD in cooked meat (c.m.)

	Mean recovery ^a ± RSD ^b			R ²	Linearity range ^c	LOD ^{c,d}	LOQ ^{c,e}
	Level 1 ^c 25	Level 2 ^c 100	Level 3 ^c 200				
CLD	84 ± 2%	102 ± 5%	99 ± 4%	0.9997	6–220	1.9	5.8

a: n = 10; b: Relative Standard Deviation; c: ng CLD.g⁻¹c.m.; d: Limit Of Detection; e: Limit Of Quantification.

was weighed before and after the cooking process in order to measure the average weight loss (Eq. (1)). The same conditions (time, temperature, quantity of peanut oil) were applied whatever the type of meat (liver, kidney, rib, chuck, top-sirloin and sirloin) in order to facilitate the interpretation of results and to allow comparison between the experiments. Hence, the “pan” process took 10 min at 210 °C (temperature measured from the pan itself) using 10 mL of peanut oil. The “oven” process took 20 min at 200 °C (thermostat 5) using 10 mL of peanut oil and the meat was covered by aluminium foil. The “microwave” process took 5 min at 750 W. The “grill” process took 10 min at 450 W.

The experiments were conducted in triplicate to evaluate repeatability.

Average weight loss (%)

$$= \frac{\text{weight of uncooked matrix (g)} - \text{weight of cooked matrix (g)}}{\text{weight of uncooked matrix (g)}} \times 100 \quad (1)$$

2.4. Extraction and analysis

2.4.1. Extraction procedure

The extraction procedure was based on previous research (Saint-Hilaire et al., 2018) and adapted to the present study. The whole piece of meat (whether cooked or uncooked) was ground for 1 min at 7000 rpm with the GM 200 Grindomix. Four grams (4.0 g ± 0.1 g) of the ground meat were placed in a 50 mL centrifuge tube before adding 6 mL of ultrapure water, then homogenised with the IKA Ultra-Turrax T25 homogeniser at 5000 rpm for 3 min. The sample was stirred with the Genie 2 vortex mixer for 1 min before adding 50 µL of internal standard at 5 µg mL⁻¹ to the sample. The mixture was vortexed for 30 s and kept in contact for one night in the fridge (4 °C). The next day, 10 mL of acetonitrile was added and the tube was stirred for 1 min before and after addition of the QuEChERS extraction kit. The tube was then centrifuged at 4000 rpm for 5 min with an Eppendorf centrifuge 5810. Six millilitres of the supernatant acetonitrile phase was collected and placed in a 15 mL centrifuge tube containing 150 mg of PSA, 150 mg of C18 and 900 mg of MgSO₄. The tube was vortexed for 1 min and then centrifuged at 4000 rpm for 5 min. Next, 1.5 mL of the supernatant was deposited in a 2 mL amber vial before injection into the HPLC-MS/MS and HPLC-HRMS systems.

2.4.2. HPLC-MS/MS conditions

The analytical conditions used in the present study were the same as previously described by Saint-Hilaire et al. (2018). The following conditions were used for the HPLC-MS/MS analysis: a gradient using Milli-Q water (Solvent A) and acetonitrile (Solvent B), each containing 0.1% formic acid, was used at a constant flow rate of 0.2 mL.min⁻¹. The gradient started with 60% of solvent B and was held for 5 min, then increased to 98% of solvent B over the next 4 min. The gradient was held for 11 min and then decreased to 60% of solvent B for 5 min. The injection volume was 5 µL and the column temperature was set at 40 °C. The detection was carried out in negative ionisation mode (ESI⁻). The ion spray voltage of the 5500 Q-trap mass spectrometer was set at -4.5 kV in ESI. The source temperature was set at 600 °C and nitrogen

was used as the desolvation gas. The 5500 Q-trap mass spectrometer was used in MRM, and the collision energy (CE) and the collision cell exit potential (CXP) were optimised for CLD and ¹³C₈-CLD with individual infusion solutions (Table SD-2).

Analyst 1.5.1 software (AB Sciex, Les Ulis, France) was used for equipment control and data acquisition.

According to European Union guidelines on the analysis of pesticide residues, SANTE/11813/2017 (European Commission, 2017), two consecutive single reaction monitoring (SRM) runs were performed (506.7/426.7 and 508.7/428.7). The two identification criteria are a retention time deviation of plus or minus 0.1 min, and a difference between both SRM ratios of less than 30%.

The performance of the analytical method developed to measure CLD in fresh meat (f.m.) is already displayed in the article of Saint-Hilaire et al. (2018) with a LOQ of 1.36 ng CLD.g⁻¹f.m.

The performance of the same analytical method applied to measure CLD in cooked meat (c.m.) is mentioned in Table 1 and are expressed in ng CLD.g⁻¹c.m. The instrumental response linearity obtained (5, 10, 50, 100, 150, 200 and 250 ng CLD.mL⁻¹) with CLD in acetonitrile and with a constant concentration of ¹³C₈-CLD (90 ng mL⁻¹) was satisfactory (R² = 0.9997). Accuracy and repeatability were evaluated by analysing the target compound at three different concentrations (25, 100 and 200 ng CLD.g⁻¹c.m.) in cooked meat. The relative standard deviation (%RSD) ranged from 2% to 5% due to the isotopic dilution, indicating excellent repeatability. The mean recovery values of CLD in cooked meat were between 80% and 120% (Table 1). The limit of detection (LOD) of CLD, which was calculated by multiplying by three the standard deviation of ten replicates of cooked meat spiked (over one night of contact time) at the lowest concentration (25 ng CLD.g⁻¹c.m.), was 1.9 ng CLD.g⁻¹c.m.. The LOQ in cooked meat (5.8 ng CLD.g⁻¹c.m.) was set as three times the LOD (Table 1). All of these data meet the requirements established by the SANTE/EU guidelines (European Commission, 2017).

2.4.3. HPLC-HRMS/MS conditions

The following conditions were used for the HPLC-HRMS/MS analysis: a gradient using Milli-Q water (Solvent A) and acetonitrile (Solvent B) was used at a constant flow rate of 0.350 mL.min⁻¹. The gradient started after 3 min of equilibrium with 10% of solvent B and was then increased to 100% of solvent B over the next 30 min. The gradient was held for 5 min and then decreased to 10% of solvent B over 1 min. The injection volume was 5.0 µL and the column temperature was set at 40 °C. The detection was carried out in both negative (ESI⁻) and positive (ESI⁺) ionisation modes. The mass parameters are listed in Table SD-3. Analyst 1.5.1 software (AB Sciex, Les Ulis, France) was used for equipment control and data acquisition.

2.5. Data processing

All the experiments were conducted in triplicate and the raw data are available in Table SD-4A and B. According to the situations, the corresponding triplicate values or median values were used.

As the concentrations measured in cooked meat were expressed in ng CLD.g⁻¹c.m., it has been necessary to convert these values in ng CLD.g⁻¹f.m. by using the following equation (Eq. (2)) to allow a direct

comparison between the CLD concentration in raw and cooked meat taking into account the weight loss that occurs during the cooking process:

$$\text{Concentration ng CLD. g}^{-1} \text{ f. m.} = (\text{concentration ng CLD. g}^{-1} \text{ of c. m.}) * (1 - \text{average weight loss}) \quad (2)$$

2.5.1. Processing factors

CLD residue reduction or concentration during the four home cooking processes was evaluated by calculating the processing factor (PF) with the help of Equation (3) (Eq. (3)):

$$\text{PF}(\%) = C_{\text{after}}/C_{\text{before}} * 100 \quad (3)$$

with C_{after} and C_{before} the median concentration of CLD after and before the cooking process respectively (Huan et al., 2015; OCDE/OECD, 2008) both expressed in ng CLD.g⁻¹f.m. A PF value below 100% indicates a reduction of residues. On the contrary, a PF value equal to 100% means no change, and a PF value greater than 100% indicates a concentration of CLD in the matrix (Huan et al., 2015).

2.5.2. Multivariate analysis

A principal component analysis (PCA) was used to describe the main tendencies in the data set. The input data used for the PCA were first normalised (centred and reduced). PCA turns original variables into new variables i.e. principal components (PCs), which are orthogonal and not inter-correlated. The PCs were ordered in such a way that the first components represent the largest fraction of the total data variance. A loading plot and a score plot were generated that represent respectively the correlations between the variables and the similarities/dissimilarities between samples (Parinet et al., 2010). The PCA was carried out using the FactoMineR package and Rstudio 1.2 software.

2.5.3. Univariate analysis

The Shapiro-Wilk test was carried out to check data distribution. According to the previous results, a Friedman test, Nemenyi (post-hoc Friedman) test, paired-Wilcoxon test or a one-way ANOVA test and paired *t*-test were carried out in order to observe any impact of cooking processes on CLD content in beef. The Rstudio 1.2 software was used for univariate analysis.

2.5.4. Target and suspect screening with HPLC-HRMS/MS

The identification and/or annotation criteria were based on the identification levels proposed by Schymanski et al. (2014).

For target screening, the identification of CLD and 5b-hydro-CLD was based on the following parameters and their tolerance values: retention time (tolerance of 0.5 min), exact mass error (tolerance of 10 ppm if the area was less than or equal to 500 arbitrary units (a.u.) and a tolerance of 5 ppm if the area was greater than 500 a.u.), a perfect match between the expected and obtained isotopic profile and MS² spectra.

For suspect screening, a list of 43 compounds of CLD by-products and metabolites was used, based on a CLD literature review (Chevallier et al., 2019; Dolfing et al., 2012; Macarie et al., 2016) (Table SD-5). The same criteria as for target screening were employed, the only difference being that there was no comparison between the expected and obtained chromatographic retention time (Rt).

Sample data were processed by Masterview 1.1 software (AB Sciex, Les Ulis, France).

2.5.5. Isotopic profile pattern and mass defect approaches

Cl³⁵ and Cl³⁷ are the only two stable isotopes of the chlorine atom and are naturally present on Earth (75.77% and 24.23% abundance respectively). Chlorinated compounds have a typical isotopic profile, which allows their clear identification based on their percentage of presence and the 1.997 *m/z* difference between them (Andra et al.,

2017).

Mass defect (MD) is another approach characterised by the calculation of the difference between the nominal mass (rounded mass) and the exact mass of an atom or compound. The MD approach is an effective non-target approach for finding the metabolites of a compound, as all of them have close MD values. This approach is also used to find halogenated compounds in environment samples (Léon et al., 2019; Pan et al., 2017; Ubukata et al., 2015). Chlorinated compounds are hypothesised to lose a chlorine atom during their degradation, which is replaced by a hydrogen atom. The nominal difference due to this degradation process equals 34 Da (*m* – Cl + H) and the exact difference 33.96102 Da. All the *m/z* values (features) are normalised by multiplying them by 34/33.96102. The graph used to find the metabolites shows the H/Cl normalised mass defect along the Y axis as a function of the H/Cl normalised nominal mass along the X axis. All the CLD metabolites should have a slight variation in mass defect, creating a line of dots on this graph (Ubukata et al., 2015).

HaloSeeker 1.0 is open source software that uses the isotopic profile (IP) and MD approaches to search for and identify halogenated compounds (chlorine and bromine) and metabolites/by-products present in the HRMS spectra (Léon et al., 2019). Before being processed by HaloSeeker 1.0, HRMS centroid spectra and their corresponding data have to be converted into a universal format (.MzXML) using the Proteowizard software (<http://proteowizard.sourceforge.net>). HaloSeeker 1.0 extracts features (i.e. a pair made up of an *m/z* value and chromatographic retention time) that belong to the same compound and clusters them. It also offers an intuitive interface and H/Cl mass defect graph in order to find metabolites and halogenated compounds. In our case, the following conditions were used: polarity of the ionisation = negative; *m/z* tolerance = 10 ppm; peak width = 5 to 60 s; prefilter step = 0; prefilter level = 0; sntresh = 0; Rt tolerance = 1 s; *m/z* tolerance = 0.5 mDa; Rt min = 1.5 min; pairing & ratio filter = F1; slope filter = all or 100% Cl.

2.5.6. In silico annotation

Original equipment manufacturers such as Sciex offer with the HRMS itself diverse software to help with the identification/annotation step. The Formula Finder algorithm, which is part of the MasterView software (Sciex), tries to predict the possible chemical formula based on the MS and MS/MS spectrum using the precursor ion's mass accuracy, isotopic pattern and MS/MS fragmentation pattern. Nevertheless, a high formula finder score does not always mean that the identified compound is actually the compound in the sample. To set the algorithm, prior to annotation the user should determine the range of numbers for C, H, N, O and Cl (for example from 0 to 10 for C in the raw formula). In this study, the following parameters were set: H (1–50), C (1–30), O (0–10), Cl (0–10).

Open software like HaloSeeker also offers the same kind of algorithm. In order to compare the performance, efficiency and trustworthiness of these algorithms, a comparison was made between these two software programs and their annotation algorithms. The HaloSeeker parameters were thus set identically to those of Formula Finder.

The two algorithms processed all the features and feature clusters, and a comparison was made.

3. Results and discussion

3.1. Distribution of CLD in the tissue of a contaminated bovine

The CLD residue concentrations found in the 6 uncooked meat matrices seized by the authorities in Martinique and received by ANSES are presented in Table 2. Hence, the median CLD concentrations found in this animal for raw chuck, top-sirloin, sirloin, rib, kidney and liver were 53, 45, 45, 35, 163 and 645 ng.g⁻¹f.m., respectively. The concentrations in offal (kidney and liver) were the highest, as expected.

Table 2CLD concentrations (ng CLD.g⁻¹f.m.^a) in bovine matrices before (uncooked) and after various home cooking processes.

Home cooking process	Home cooking process				
	Uncooked Median (min–max)	Grill Median (min–max)	Microwave Median (min–max)	Oven Median (min–max)	Pan Median (min–max)
All matrices	49 (35–645)	30 (22–387)	4.5 (1.2–26.9)	26 (16–246)	20 (17–291)
All muscles	45 (35–53)	26 (22–31)	2.8 (1.2–5.7)	19 (16–33)	19 (17–20)
All offal	404 (163–645)	238 (88–387)	20 (13–27)	163 (81–246)	155 (20–291)
Chuck	53 (47–53)	30 (24–34)	2.4 (2.3–3.5)	33 (31–33)	20 (19–21)
Top-sirloin	45 (41–55)	31 (29–32)	3.2 (2.6–3.3)	20 (19–21)	17 (15–18)
Sirloin	45 (45–46)	23 (22–30)	1.2 (1.2–1.2)	19 (16–21)	19 (17–20)
Rib	35 (34–36)	22 (20–23)	5.7 (5.2–6.1)	16 (15–18)	20 (19–21)
Kidney	163 (139–167)	88 (83–89)	27 (24–43)	81 (75–98)	20 (20–21)
Liver	645 (602–670)	387 (351–428)	13 (10–19)	246 (239–275)	291 (239–297)

a: fresh meat; for the cooked meat f.m. means “equivalent to raw fresh meat”.

Indeed, previous studies had demonstrated the key role played by the liver in CLD metabolism (Saint-Hilaire et al., 2018). Nevertheless, the concentrations found in muscle tissues (chuck, top-sirloin, sirloin and rib) were of great interest and more concerning as people frequently consume them. Indeed, until recently CLD was considered highly lipophilic due to its chemical structure and its high Log Kow value (4.5–5.4). Nevertheless, CLD in the environment and in animal organisms is of the gem-diol form, with a polar moiety that could explain the distribution observed in live animals. It has been noticed that CLD concentrations differed according to the various muscles studied, probably due to their composition in terms of lipids, proteins and carbohydrates and their oxidative characteristics (Lastel et al., 2016). This highlights the ambiguous behaviour and distribution of CLD in animal tissues. All the concentrations measured in the muscles or offal were superior to the new MRL (20 ng.g⁻¹f.m.) set by French authorities.

3.2. Effects of home cooking processes

3.2.1. Principal component analysis

A principal component analysis (PCA) (Fig. 1) was designed to (i) differentiate the home cooking processes in terms of their impact on CLD content in matrices and to (ii) categorise the behaviour of matrices contaminated by CLD when cooked in various ways. The input for this analysis was the triplicate measurements of CLD content in the six matrices subjected to four cooking processes (Table SD-4). PC1 and PC2 represented respectively 81.3% and 18.6% of the total variance explained.

As the variable factors map (loading plot) showed (Fig. 1a variable factor map), there are two types of cooking process/variables. Indeed, pan, oven and grill processes and their corresponding triplicate measurements attributed numbers from 1 to 3 constructed PC1 with strong positive loadings. In contrast, the microwave process and its corresponding triplicate measurements constructed PC2 with a moderate-strong positive loading. Based on these observations, it can be concluded that the microwave cooking process, whatever the matrix considered, differed from the other home cooking processes (pan, oven and grill).

Fig. 1b illustrates the individuals factor map (score plot). The kidney and liver were clustered together as they were in the positive part of the PC1. In contrast, the muscle matrices were clustered in the negative part of PC1, which means that whatever the cooking process involved, the behaviour of muscles contaminated by CLD differed from that of offal (Fig. 1b_individuals factor map).

3.2.2. All matrices

To assess by univariate approaches which home cooking processes were able to significantly reduce the CLD content in the beef matrices under study, whatever the type of the matrix, the median values of all the experiments (Table SD-4) were pooled in the same data set called

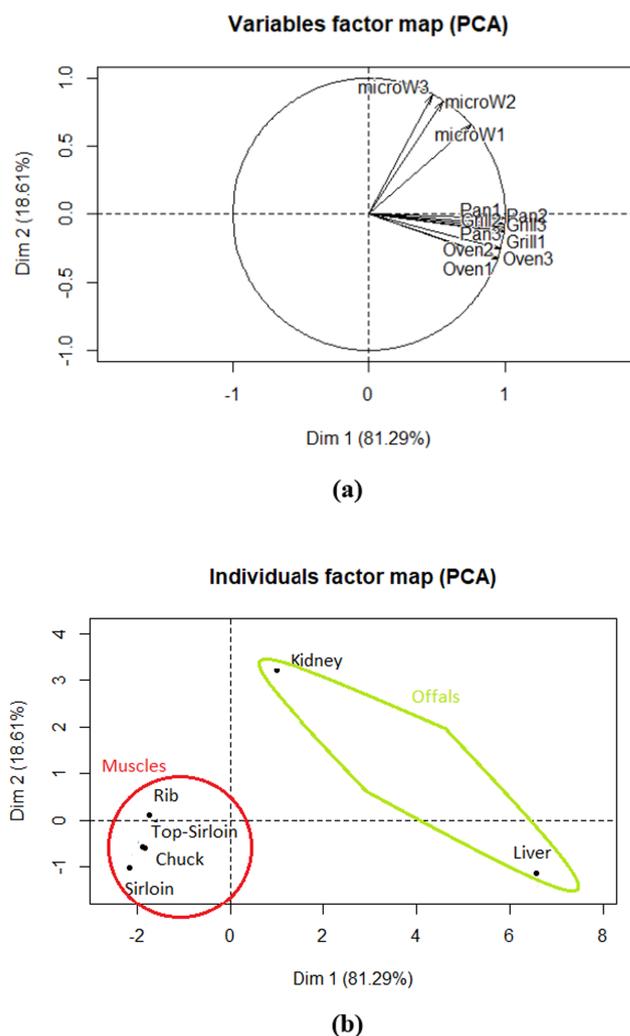


Fig. 1. Principal component analysis with all the data, (a) loading plot and (b) score plot.

“All matrices” (n = 30).

The general trend observed in Fig. 2a reveals that the boxplots are quite similar for grill, oven processes with uncooked meat with relatively similar data dispersion. In contrast, the box plot for all the matrices cooked in the microwave oven and by a pan process differed from the others as the data were less dispersed and the median CLD value were lower than the corresponding median values measured in uncooked matrices. Moreover, the median CLD values for the microwave and the pan cooking processes were under or equal to the MRL of CLD

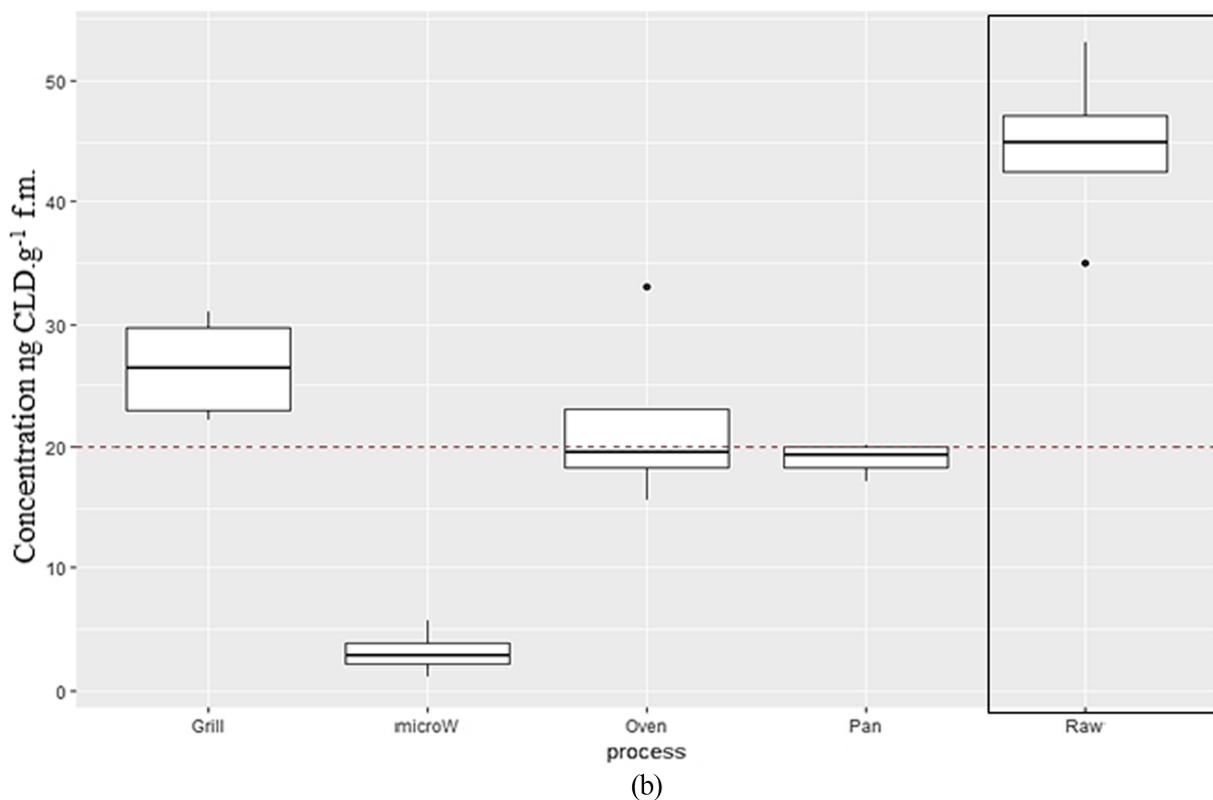
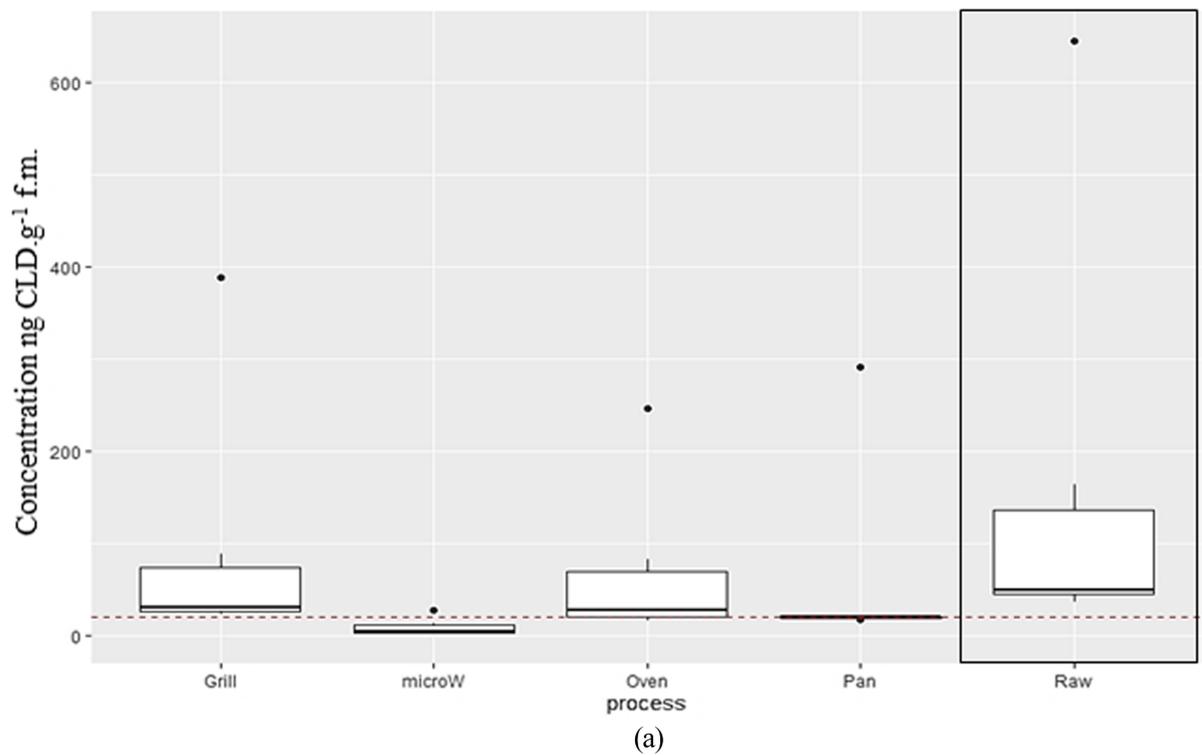


Fig. 2. Boxplots of CLD concentrations ($\text{ng CLD.g}^{-1}\text{f.m.}^a$) after various home cooking processes (grill, microwave, oven and pan) and before (raw/uncooked in the black box) - (a) for all the studied matrices, (b) for muscles and (c) for offal matrices. The red dashed line corresponds to the MRL ($20 \text{ ng.g}^{-1}\text{f.m.}$) for bovines. a: fresh meat.

in meat ($20 \text{ ng CLD.g}^{-1}\text{f.m.}$), respectively. Outliers (more than 1.5 times of upper quartile) were observed for each home cooking process and were found to correspond to the median CLD values measured in liver (Table SD-4). In the case of the microwave process, even the

outlier due to the CLD value measured in liver is close to the MRL (Fig. 2a).

The results for 'all matrices' indicated a median CLD concentration for uncooked matrices of $49 \text{ ng CLD.g}^{-1}\text{f.m.}$ and after the grill, oven

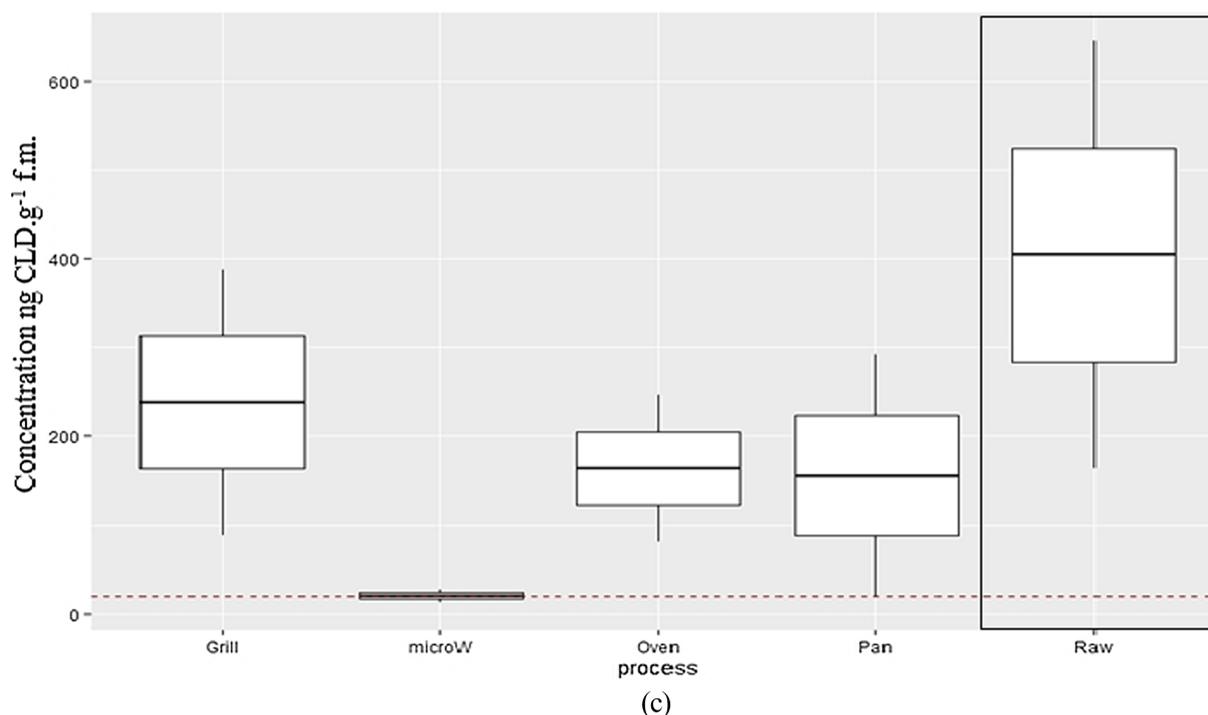


Fig. 2. (continued)

and pan processes, a decrease was observed; the median CLD concentrations were respectively 30, 26 and 20 ng CLD.g⁻¹f.m. (Table 2). The decrease was higher after microwave cooking, with a median CLD concentration of 4.5 ng CLD.g⁻¹f.m.

Based on the observations made in Fig. 2a and Table 2 (for ‘all matrices’), the CLD content in beef matrices could be reduced whatever the matrices and the cooking process. The Friedman test (Shapiro-Wilk test with p-value < 0.05) indicated a significant difference between all the cooking processes studied (p-value < 0.01). The Nemenyi test (Post-hoc of Friedman) showed that there were a significant difference in data for uncooked and microwave-cooked meat (p-value < 0.05) and between uncooked and pan-cooked meat (p-value < 0.05).

3.2.3. All muscles vs all offal

The same approach as above was used for the two datasets highlighted by the PCA: “All muscles” (n = 20) and “All offal” (n = 10) (Table 2).

For the four cooking processes, the median CLD concentrations in all muscles were lower than those measured in uncooked matrices (Fig. 2b, Table 2 “all muscles”). The lowest median value of 2.8 ng CLD.g⁻¹f.m. was obtained with the microwave process, which reduced the CLD concentration in cooked meat to below the new MRL (20 ng CLD.g⁻¹f.m.). The data were heterogeneously dispersed, being highest for the grill and oven cooking processes and lowest for the pan process (Fig. 2b). It has to be noticed the fact that the median CLD concentrations measured for oven and pan cooking processes are below the new MRL (20 ng CLD.g⁻¹f.m.).

Similarly, the median CLD concentrations in all offal were lower than those measured in the uncooked matrices for all four home cooking processes (Fig. 2c, Table 2 “all offal”). The lowest median value of 20 ng CLD.g⁻¹f.m. was also obtained by the microwave cooking process, which reduced the CLD content by a factor close to twenty times (Table 2). Data were generally highly dispersed except for the microwave cooking process (Fig. 2c).

These results also indicate that the median CLD concentration is around ten times higher in offal than in muscles (Fig. 2, Table 2), in accordance with the literature (Saint-Hilaire et al., 2018) that specifies the liver as the organ that accumulates the most CLD, and in accordance

with the PCA carried out beforehand (Lastel et al., 2016). Finally, all the cooking processes studied appear to reduce drastically CLD content, but the microwave oven is most effective.

A one-way ANOVA test on the median values for “all muscles” (n = 20) indicated a significant difference between all the cooked muscles and uncooked muscle matrices (p-value < 0.005). A paired t-test revealed a significant difference between the CLD content measured in uncooked muscle matrices and muscle matrices cooked in a grill, microwave oven, oven and fried by a pan (p-value < 0.05). For pooled offal matrices, no significant difference was observed with the Friedman test. In fact, only ten median values were used for the Friedman test, which was probably not sufficient. For this reason, the values from the experiments carried out in triplicate on offal (n = 30, Table SD-4) were used through a paired-Wilcoxon test. This test indicated two significant differences: one between uncooked offal matrices and offal matrices cooked in a microwave oven (p-value < 0.05), and a second one between uncooked offal matrices and offal matrices fried in a pan (p-value < 0.05).

3.2.4. Processing factors for each matrix

To calculate the processing factors (PFs) as defined by OECD guidelines (OCDE/OECD, 2008), each matrix was also considered individually as regards its behaviour during cooking processes. The PFs were calculated from the median values of the triplicate experiments (Tables 2 and 3). For all individual matrices, it should be noted that,

Table 3

Processing factors for CLD (%) for each matrix/cooking process pair.

		Home Cooking Process			
		Grill	Microwave	Oven	Pan
Matrix	Chuck	55	5	62	38
	Top-sirloin	69	7	44	38
	Sirloin	52	3	42	41
	Rib	63	16	45	57
	Kidney	54	17	50	12
	Liver	60	2	38	45

whatever the cooking process used, the concentrations of CLD decreased significantly after cooking them (Table 2). For the chuck matrix, the PFs calculated ranged from 5 to 62% (Table 3). Microwave cooking induced the greatest reduction of CLD content in chuck, which dropped below the MRL. Similarly, for the top-sirloin and sirloin matrices, microwave cooking induced the greatest reduction of CLD (PF of 7 and 3%, respectively), which dropped also far below the MRL. The other cooking processes although effective induced higher PFs, between 38 and 69% (Table 3) but oven and pan cooking reduced also the CLD content below the MRL. For the rib and kidney matrices, the PFs ranged from 16 to 63% and from 17 to 54% respectively. Microwave and oven cooking reduced rib's CLD content below the MRL. Finally, for the liver matrix, microwave cooking induced the greatest reduction (PF of 2%), with a CLD content below the MRL. All the other cooking processes induced an important reduction (PFs between 38 and 60%) but always above the MRL.

According to these results, microwave cooking, generally followed by pan cooking, was the most efficient process for reducing CLD content in beef matrices, with PFs ranging from 2 to 17%, whatever the matrix. The grill and oven cooking processes can also reduce, but less efficiently, the CLD content in meat. Moreover, the cooking processes probably had different impacts on CLD content depending on the matrix composition. A radar plot drawn up to look for tendencies indicated that rib meat did not react to the four cooking processes in the same way as the other matrices and seemed to be the most refractory to CLD reduction through cooking (Fig. 3). This is probably due to its composition, as it has a high proportion of lipids. Similarly, CLD levels in chuck were not really impacted by oven cooking. Otherwise, the other matrices seemed to react quite similarly (Fig. 3).

3.3. The search for CLD by-products and metabolites with HPLC-HRMS/MS

The following part was designed to investigate the production of any CLD by-products and the potential presence of organochlorine contaminants in uncooked and microwave-cooked liver, as the highest CLD concentration (of 645 ng CLD.g⁻¹) was found in uncooked liver and as the microwave cooking process had the highest impact on the reduction of CLD content in the liver, with a PF of 2%.

3.3.1. Target and suspect screening with HPLC-HRMS/MS

In the uncooked liver, CLD and 5b-hydro-CLD were identified at level one (Table 4, Fig. 4) as the retention time, isotopic profile, exact mass error on the precursor ions (506.6 and 508.6 Da) and product ions (426.7 and 428.7 Da) fit perfectly with the criteria set out in the *Material and Methods* part and in the article of Schymanski et al. (2014). The areas of the CLD precursor ions were 14,528 a. u. for ion 1 (506.6 Da) and 29,762 a. u. for ion 2 (508.6 Da) with in both cases a signal to noise ratio above 10,000. For the precursor ions of 5b-hydro-CLD, the areas were respectively 35 a. u. and 22 a. u. for ions 1 (472.7 Da) and 2 (474.7 Da). The signal to noise ratio, of 72 and 41 respectively, were lower than for CLD. These results are corroborated with the results of Stafford et al. (1978) who found a mono-hydrochlordecone (mono-hydro-CLD) obtained by photodegradation of CLD in the carcass of several wild avian. The mass spectra provided by these authors for this mono-hydro-CLD is identical to that of the mono-hydro-CLD photoproduct observed by Alley et al. (1974) and Harless et al. (1978) and clearly identified as 5b-monohydroCLD in the CAS nomenclature (= 8-monohydroCLD in IUPAC nomenclature).

In the cooked liver, only CLD was identified at level one with all the required criteria. Nevertheless, the areas of CLD precursor ions 1 and 2 were ten times lower than the corresponding values in the uncooked liver. This tendency is coherent with the results observed with the HPLC-MS/MS target approach (Table 2) and with the PF of 2% (Table 3). 5b-hydro-CLD was not detected/found (NF) in the cooked liver probably because the microwave process also reduced the molecule's content due to volatilisation or degradation processes. The similarities between CLD and 5b-hydro-CLD structures could also explain this observation.

Suspect screening was also carried out on uncooked and cooked liver using a list of known CLD metabolites and by-products based on a literature review (Table SD-5). For suspect screening identification/annotation, the same criteria as those mentioned previously were used, except the chromatographic retention times which were unknown by definition.

This approach detected a mono-hydrochlordecone isomer different from 5b-hydro-CLD in the uncooked liver at a chromatographic retention time of 19.5 min (Fig. 4 and Table 4). The retention time, the exact mass error, the MS spectra and MS² spectra listed in Table 4 of the

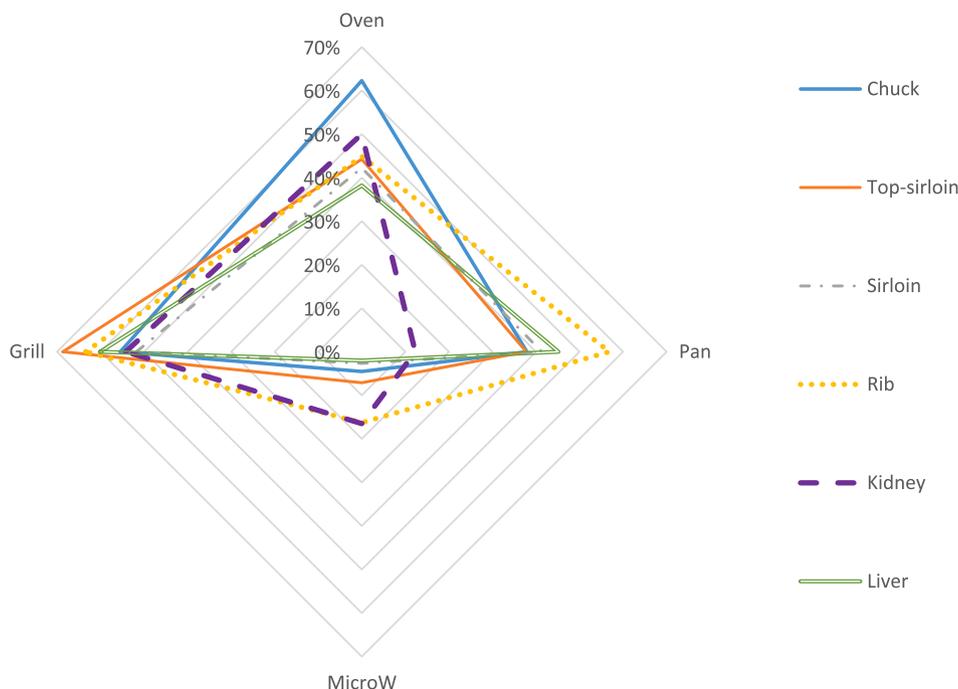


Fig. 3. Radar plot – PF (%) for each matrix and home cooking process.

Table 4
Compounds annotated or identified by target and suspect screening in the HPLC-FRMS/MS spectra with negative mode ionisation (ESI⁻).

Matrix	Type of screening	Compound	Identification criteria ^a	Area & S/N	Level of identification
Uncooked Liver	Target	CLD	Em precursor ion 1 (506.6 Da) = -0.5 ppm;	Em precursor ion 1 (506.6 Da) area = 14,528 a.u.;	1
			Em precursor ion 2 (508.6 Da) = 0 ppm;	Em precursor ion 1 (506.6 Da) S/N = 29,762;	
			Em product ion 1 (426.7) = -0.2 ppm;	Em precursor ion 2 (508.6 Da) area = 12,231 a.u.;	
			Em product ion 2 (428.7) = -0.9 ppm;	Em precursor ion 2 (508.6 Da) S/N = 14,286;	
			Rt = 20.8 min;	Em product ion 1 (426.7) area = 7821 a.u.;	
			IP = ok;	Em product ion 1 (426.7) S/N = 12,483;	
				Em product ion 2 (428.7) area = 5830 a.u.;	
				Em product ion 2 (428.7) S/N = 10,295;	
				Em precursor ion 1 (472.7 Da) = 0.9 ppm;	
				Em precursor ion 2 (474.7 Da) = -10.9 ppm;	
5b-hydro-CLD			Em product ion 1 (392.7) = -1.6 ppm;	Em precursor ion 1 (472.7 Da) area = 35 a.u.;	1
			Em product ion 2 (394.7) = NF;	Em precursor ion 1 (472.7 Da) S/N = 72;	
			Rt = 18.5 min;	Em precursor ion 2 (474.7 Da) area = 22 a.u.;	
			IP = ok;	Em precursor ion 2 (474.7 Da) S/N = 41;	
				Em product ion 1 (392.7) area = 22 a.u.;	
				Em product ion 1 (392.7) S/N = 40;	
				Em product ion 2 (394.7) area = NF;	
				Em product ion 2 (394.7) S/N = NF;	
				Em precursor ion 1 (472.7 Da) = -6 ppm;	
				Em precursor ion 2 (474.7 Da) = -6.6 ppm;	
Suspect		Monohydro-CLD	Em product ion 1 (392.7) = -4.7 ppm;	Em precursor ion 1 (472.7 Da) area = 138 a.u.;	2a
			Em product ion 2 (394.7) = -5.7 ppm;	Em precursor ion 1 (472.7 Da) S/N = 182;	
			Rt = 19.5 min;	Em precursor ion 2 (474.7 Da) area = 108 a.u.;	
			IP = ok;	Em precursor ion 2 (474.7 Da) S/N = 157;	
				Em product ion 1 (392.7) area = 60 a.u.;	
				Em product ion 1 (392.7) S/N = 71;	
				Em product ion 2 (394.7) area = 31 a.u.;	
				Em product ion 2 (394.7) S/N = 68;	
				Em precursor ion 1 (506.6 Da) = -0.7 ppm;	
				Em precursor ion 2 (508.6 Da) = -1.3 ppm;	
Microwaved Liver	Target	CLD	Em product ion 1 (426.7) = -1.5 ppm;	Em precursor ion 1 (506.6 Da) area = 1648 a.u.;	1
			Em product ion 2 (428.7) = -1.9 ppm;	Em precursor ion 2 (508.6 Da) S/N = 22,726;	
			Rt = 20.8 min;	Em precursor ion 2 (508.6 Da) area = 1411 a.u.;	
			IP = ok;	Em precursor ion 2 (508.6 Da) S/N = 24,800;	
				Em product ion 1 (426.7) area = 887 a.u.;	
				Em product ion 1 (426.7) S/N = 3354;	
				Em product ion 2 (428.7) area = 649 a.u.;	
				Em product ion 2 (428.7) S/N = 3455;	
				NF	
				NF	
Suspect		5b-hydro-CLD Monohydro-CLD	NF	NF	NF
			NF	NF	NF

^a Em: error on exact mass; Rt: chromatographic retention time; IP: isotopic profile similitude; A: area; S/N: signal to noise; NF: not found.

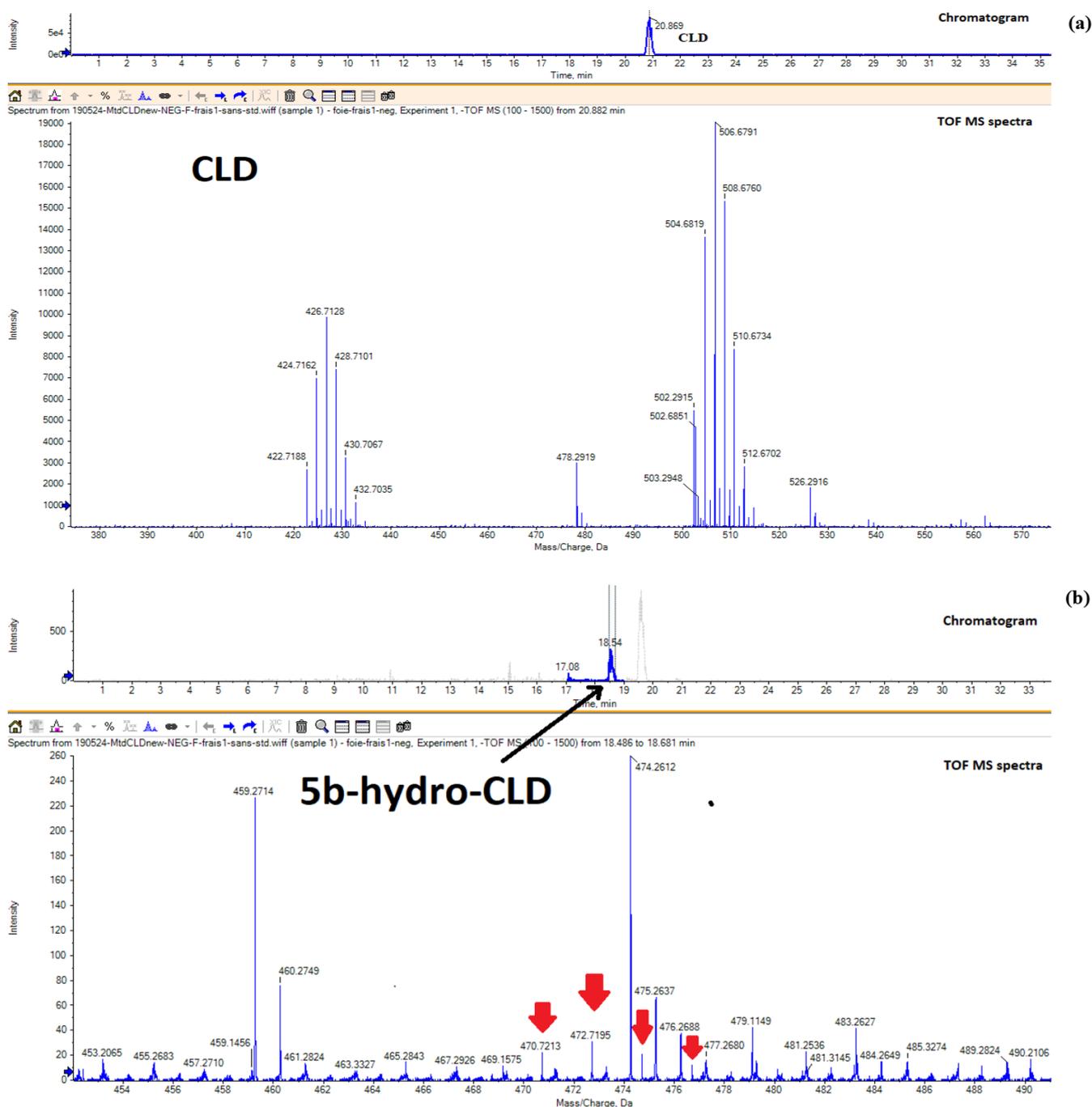


Fig. 4. Extracted ion chromatogram (EIC) and TOF MS spectra for uncooked bovine liver (a) chlordecone (CLD), (b) 5b-hydrochlordecone (5b-hydro-CLD), (c) a mono-hydrochlordecone (mono-hydro-CLD) and (d) 5b-hydro-CLD standard used to spike samples.

suspected structure are coherent with the theoretical raw formula ($C_{10}Cl_9O_2H_3$ – gem-diol form/ $C_{10}Cl_9OH$ – ketone form). As no standard was available, it was not feasible to confirm this assumption. For this reason, a level of identification/annotation of 2a has been decided upon for these features. Moreover, the area for precursor ion 1 (472.7 Da at 19.5 min) is five times higher than the value for 5b-hydro-CLD, 138 a. u. vs. 35 a. u. so points to a concentration probably five times higher. Nevertheless, the signals/area obtained for the 5b-hydro-CLD and mono-hydro-CLD are far from the area found for CLD, which is by far the greatest contaminant in bovine liver.

No trace of 5b-hydro-CLD and/or mono-hydro-CLD was found in microwaved liver, probably due to the effect of the microwave cooking process, which, like for CLD, decreases these contaminants by a factor

of approximately ten.

3.3.2. Isotopic profile and mass defect approach with non-target screening

The uncooked liver, microwaved liver and experimental blank (sample not containing the analyte of interest, but having followed the whole analysis process) were processed using the isotopic profile and mass defect approach through the Haloseeker software. The objective was to use this blind/non-target approach to look for organochlorine compounds in the samples being studied to detect any CLD metabolites and microwaved by-products.

For the uncooked liver, either all the features were kept (Fig. 5a and Table SD-6) or only chlorine compounds were retained (100% Cl) (Fig. 5b and Table SD-6). A list of features belonging to CLD

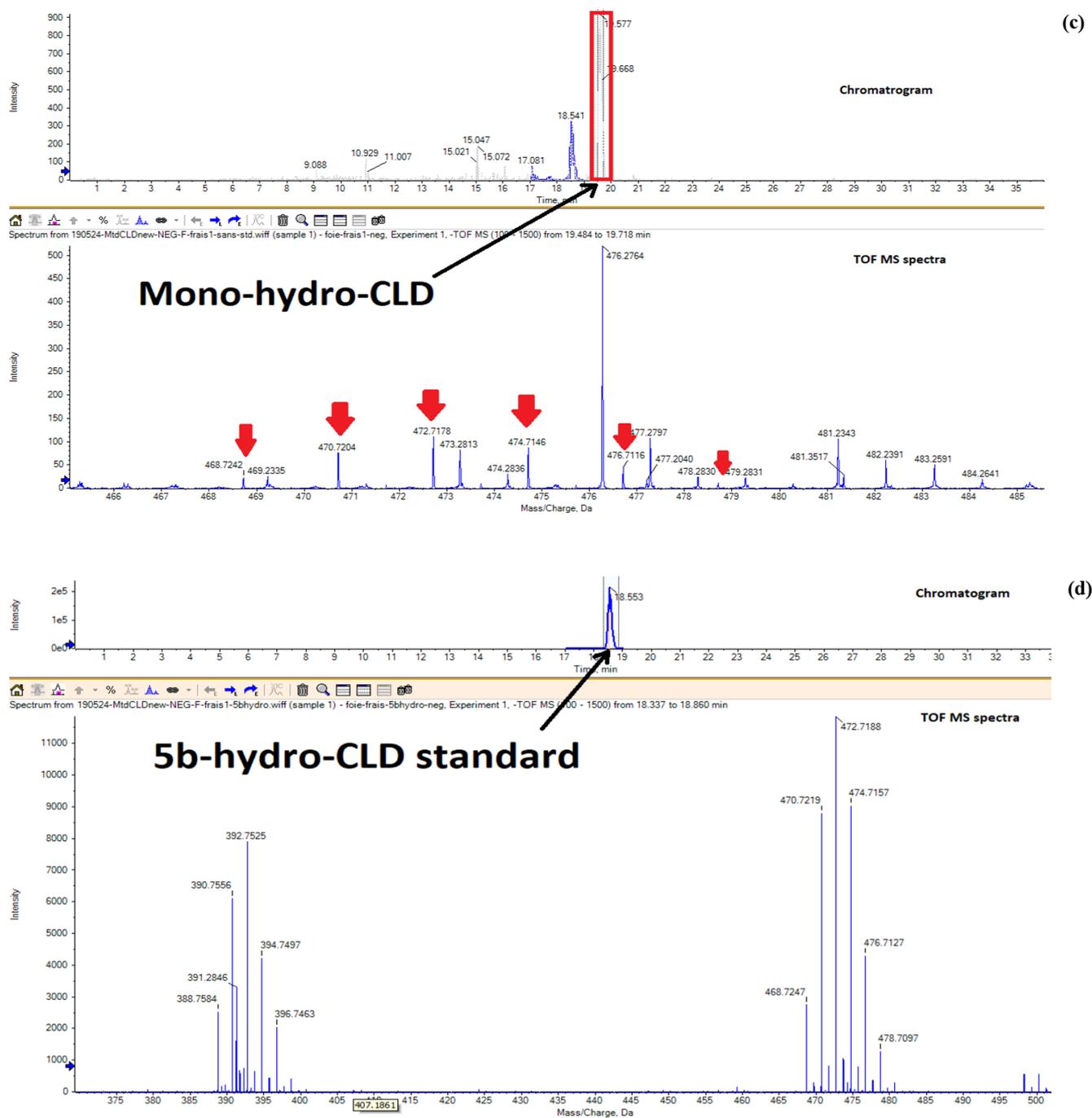


Fig. 4. (continued)

($C_{10}HCl_{10}O_2$) and to the mono-hydro-CLD were found and clustered by a red ellipse in Fig. 5a. All the isotopes, adducts and in-source fragments of CLD are grouped into clusters 2, 7, 13, 20, 40, 57, 69 and 115, as CLD is fragmented in the electrospray source (ESI) with a loss of 80 Da (cluster 7, Table 4) (Saint-Hilaire et al., 2018). The mono-hydro-CLD clusters were clusters 56 (474.6 and 476.6 Da at 19 min) and 100 (390.7, 392.7 and 394.7 Da at 19 min) with the same in-source fragmentation as CLD and the loss of 80 Da. This approach revealed no trace of 5b-hydro-CLD, even when all the features were kept. Moreover, when only the organochlorine compounds were retained (100% Cl), even mono-hydro-CLD disappeared from Fig. 5b and Table 4 as the software was not able to “consider” that the corresponding clusters/features belonged to an organochlorine compound. Only CLD therefore remained. The features that are not in the red ellipse and that do not

belong to CLD and/or 5b-hydro-CLD came from the QuEChERS salts, d-spe and from artefacts, as they were also found in the experimental blank (Fig. 5e and 5f).

The same kind of feature filtration was applied to the microwaved liver. In Fig. 5c and Table SD-6, a list of features that belong only to CLD ($C_{10}HCl_{10}O_2$) were found and grouped into clusters 30 and 43. This shows that no trace of mono-hydro-CLD or 5b-hydro-CLD was found, even when all the features were kept. Moreover, when only the organochlorine compounds were retained (100% Cl), only cluster 43 remains, this being the in-source fragment of CLD. These results are coherent with our prior results: the reduction in CLD content and the disappearance of mono-hydro-CLD during the microwave cooking process. Nevertheless, these results also show that this non-target approach with the 100% organochlorine filter misses some interesting

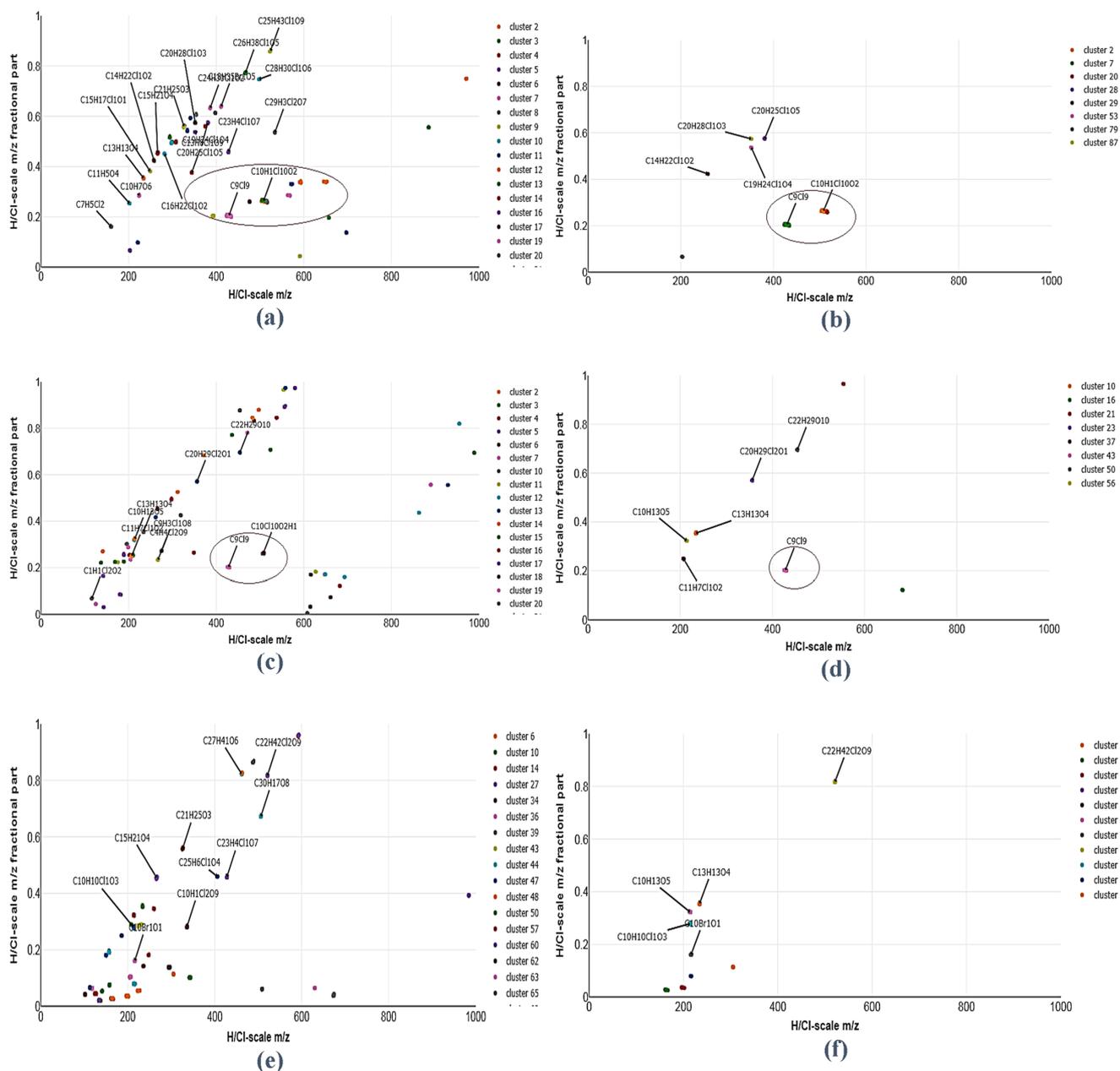


Fig. 5. H/Cl mass defect graph produced using Haloseeker: (a) Uncooked liver with all extracted features; (b) Uncooked liver with only chlorinated features; (c) Microwaved liver with all extracted features; (d) Microwaved liver with only chlorinated features; (e) Experimental blank with all extracted features; (f) Experimental blank with only chlorinated features.

features/clusters from organochlorine compounds. This is probably due to the low levels/intensity of 5b-hydro-CLD in the uncooked liver. Despite the results given above, it is interesting to observe that this untargeted approach does not align any new features with CLD, which could mean that the microwaved cooking process does not produce any CLD by-products. The features not in the red ellipse and not belonging to CLD and/or 5b-hydro-CLD came from the QuEChERS salts and d-spe and from artefacts, as they were also found in the experimental blanks (Fig. 5e and 5f).

3.3.3. *In silico* annotation comparison

The Haloseeker and MasterView (Formula Finder) annotation algorithms' ability to propose a pertinent raw formula with a relevant score was compared. The features of CLD or 5b-hydro-CLD and their corresponding standards were used as references for uncooked and microwaved livers as it was certain that these compounds were present

in the studied samples. To produce the annotation, two common lists of features ($m/z - Rt$) with a chromatographic retention time over 1.5 min originally obtained by the Haloseeker software from the uncooked and microwaved liver were used by both software programs (Table SD-6). Moreover, only chlorinated compounds were selected for the annotation. All the results are displayed in Table SD-6 and Fig. 5b and d.

In the uncooked liver, where the concentrations of CLD and 5b-hydro-CLD are the highest, the annotation and selection algorithm used by Haloseeker was not able to find a raw formula for the features belonging to the CLD clusters (Fig. 5b). However, if the operator set the right raw formulas ($C_{10}Cl_{10}O_2H_2$, C_9HCl_9) for CLD's features (clusters 2 and 7), the Haloseeker algorithm was able to propose a matching score of 90% and 98% respectively, which is quite high. On the other hand, the Formula Finder algorithm of MasterView was able to propose the right raw formulas but with a matching score of only 63% and 64%, which is only average if not to say poor considering the concentration

of CLD in the matrix ($645 \text{ ng} \cdot \text{g}^{-1}$) and the intensity/area of the features (506.6 Da and 426.7 Da with areas equal to $14,528 \text{ a. u.}$ and 7821 a. u. , Table 4) analysed by HPLC-HRMS/MS. It was not possible to learn anything from clusters 20, 28, 29, 53, 79 and 87 (Table SD-6) and the corresponding annotations proposed by both algorithms as the corresponding compounds were not available as standards and not known by the operators. Nevertheless, the annotations proposed by both algorithms are different. Haloseeker suggested chlorinated raw formulas and Formula Finder was unable to propose even one raw formula (clusters 28, 29, 79 and 87).

In the microwaved liver, where the concentration of CLD was twenty times lower than in the uncooked liver, only the in-source fragment of CLD was detected by Haloseeker (cluster 43, C_9HCl_9). Haloseeker was not able to find by itself the right raw formula. If the right raw formula was entered in the algorithm, however, Haloseeker proposed a matching score of 58%. This is quite average but consistent with the relatively weak signal (887 a. u. for product ion $1-426.7 \text{ Da}$). In contrast, for the same cluster of features, Formula Finder proposed by itself another inappropriate raw formula ($\text{C}_8\text{HCl}_9\text{O}$), with a moderate score of 57%. It was not possible to draw a conclusion on the advantage of one algorithm over the other for the other features mentioned. For the microwaved liver, Formula Finder nevertheless proposed fewer annotations than Haloseeker.

Based on this study and the application of these algorithms to real samples, it appears difficult to blindly trust the annotations they propose, especially for suspect screening. An extensive study with more compounds at different concentrations should be carried out to determine the conditions under which these algorithms can be considered reliable or not.

4. Conclusion

CLD is not equally distributed in the tissues of the bovine under study. As expected, the highest concentrations were found in the liver and kidney. Concentrations in the muscles were lower, but still above the MRL and differed from one type of muscle to another. The differences in CLD concentration between all the muscles investigated are probably due to the composition of the matrices and their oxidative character. The four home cooking processes clearly affect the CLD content in the bovine matrices under study at various levels, with PFs ranging from 2 to 69%. Microwave cooking is the most effective in reducing CLD content whatever the matrix considered, with PFs ranging from 2 to 17%. The target and suspect screening with HPLC-HRMS/MS demonstrated the capacity and versatility of HRMS instruments to detect CLD and its metabolites, especially 5b-hydro-CLD and another mono-hydro-CLD in the uncooked liver. This is the first time that the last mentioned compound has been reported in meat. Applied to liver, the non-target approach, using the isotopic profile and mass defect with dedicated software, also reveals the presence of CLD and a mono-hydro-CLD in the uncooked liver, but not the presence of 5b-hydro-CLD. This is probably due to the low intensities of the corresponding features. In microwaved liver, neither the suspect nor the non-target approaches revealed any organochlorine features/clusters. This reinforces the hypothesis that CLD and its potential by-products are volatilised during the cooking process. Nevertheless, the same approaches should be developed with the help of GC-HRMS and GC-MS/MS in order to try to detect any other volatile metabolites and by-products.

Due to the high concern of CLD content in food, from a public health point of view, this study should be extended to other food categories (pork, sheep, goats, avian, fish and seafood...) to better characterise occurrence levels of CLD after cooking.

CRedit authorship contribution statement

Deborah Martin: Investigation, Data curation. **Fiona Lobo:**

Investigation, Data curation. **Gwenaëlle Lavison-Bompard:** Resources. **Thierry Guérin:** Validation, Writing - review & editing. **Julien Parinet:** Conceptualization, Investigation, Formal analysis, Writing - original draft, Funding acquisition, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106077>.

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