Journal of Hazardous Materials 459 (2023) 132332

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Chlorinated organic compounds in concrete as specific markers for chlorine gas exposure

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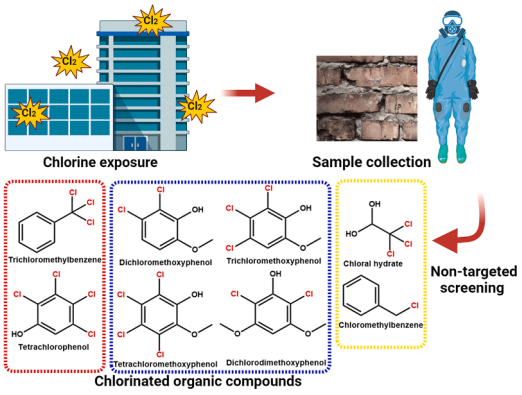
HIGHLIGHTS

- Chlorinated markers were detected by non-targeted analysis after chlorine exposure.
- Tetrachlorophenol and trichloromethylbenzene are main markers of neat chlorine gas.
- The concrete samples from the 1930s and 1950s had the most chlorinated compounds.
- Lignin or lignin degradation products are probable phenolic precursors in concrete.
- Multivariate data analysis shows distinct patterns for bleach and chlorine exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dr. C. Baiyang

Keywords:
Chlorine
Concrete of various ages and different origins
Chlorinated organic compounds
Gas Chromatography High-Resolution Mass Spectrometry (GC-HRMS)
Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS)

ABSTRACT

The formation of chlorinated organic compounds in concrete debris exposed to reactive chlorine was studied to search for markers specific to chlorine gas exposure. Concrete materials of different origins were exposed to a range of species of reactive chlorine including bleach, humid and dry chlorine gas at different concentrations. Chlorinated organic compounds in concrete extracts were analysed by targeted gas and liquid chromatography-tandem mass spectrometry (GC-MS/MS and LC-MS/MS) and by non-targeted screening using the corresponding high-resolution techniques (GC-HRMS and LC-HRMS). Overall, different levels and species of chlorinated organic compounds namely chlorophenols, chlorobenzenes, chloromethoxyphenols, chloromethylbenzenes and chloral hydrate were identified in these chlorinated concrete extracts; two examples of diagnostic markers for neat chlorine exposure were trichloromethylbenzene and tetrachlorophenol. The old concrete samples from the 1930s and 1950s had the most chlorinated organic compounds after exposure to neat chlorine gas. Lignin or lignin degradation products were identified as probable candidates for phenolic precursor molecules in the concrete samples. Multivariate data analysis (OPLS-DA) shows distinct patterns for bleach and chlorine exposure. The

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https://doi.org/10.1016/j.jhazmat.2023.132332
Received 21 June 2023; Received in revised form 4 August 2023; Accepted 15 August 2023
Available online 16 August 2023

chlorinated chemicals and specific markers for chlorine gas discovered in our research assist other laboratories in forensic investigations of chlorine gas attacks.

1. Introduction

Chlorine is a toxic chemical, produced in large scales by the electrolytic reduction of calcium chloride (brine). It has dual use for both civil and military purposes. For example, it has an important civil application as a disinfectant of drinking water, thereby protecting human health worldwide [1]. Chlorine is also vital to the chemical industry for the production of plastics, drugs, microchips, and many other products [1]. The total chlorine market is approximately 70 million tons annually, including liquefied compressed chlorine [2].

Chlorine also has a history of being used as a chemical weapon in armed conflicts. On April 22, 1915 during World War 1 (WWI), chlorine gas was used for the first time as a chemical warfare agent in Ypres, Belgium. German troops discharged 5,730 cylinders capable of releasing 180,000 kg of chlorine gas into the wind toward the allied troops [1]. The heavy chlorine gas accumulated in the trenches of Ypres, leading to devastating effects due to the lack of personal protective equipment. In the decades after WWI, a range of dedicated chemical warfare agents such as sulfur mustard, sarin, soman and VX were developed or produced e.g., by Germany, France, Russia, as well as the United States and the United Kingdom [3]. Since these agents have much higher toxicity than chlorine, it was not expected that the use of chlorine as a chemical warfare agent would re-emerge in the 21st century. In spite of the historical use as a chemical weapon, the wide range of chlorine uses in civil applications made it difficult to include in the schedules of toxic chemicals outlined in the Annexes of the Chemical Weapons Convention (CWC). However, the use of chlorine as a chemical weapon is prohibited by the CWC as defined in the general purpose criteria (Article II) [4]. More recently, chlorine was used in suicide chemical attacks in Iraq, when trucks loaded with explosive and chlorine cylinders burst in close proximity to police posts. The alleged use of chlorine as a chemical weapon in the ongoing conflict of the Syrian Arab Republic was documented in recent reports of the Organization for the Prohibition of Chemical Weapons (OPCW) [5,6]. Additionally, in a summary report by the German Public Policy Institute on the use of chemical weapons in The Syrian Arab Republic during 2013–2018, it was concluded that chlorine was indeed the toxic chemical used in 89% of the incidents reported [7]. The report concluded that the challenge of verifying the release of chlorine creates an impunity for its use as a chemical weapon.

Chlorine is a greenish-yellow diatomic gas (Cl_2) having a pungent, irritating odor, and can be suffocating if inhaled – death can occur after 30 minutes of exposure to concentrations of 430 ppm [8,9], and within a few minutes when exposed to concentrations of 1,000 ppm or above [10]. Chlorine is classified as a pulmonary irritant and one of the strongest oxidizing agents [11]. It is not normally detected in the environment because it is highly reactive by rapidly transforming into various compounds [9]. Chlorine also quickly decomposes in sunlight within minutes, and when dissolved in water, it is converted into chloride ions (Cl^-), hydrochloric (HCl), and hypochlorous (HOCl) acids [1,9]. The reaction between chlorine and sodium hydroxide produces sodium hypochlorite, the active ingredient in commercial bleach products.

When chlorine is released into the environment, it reacts with both organic and inorganic chemicals that it contacts, thereby producing chlorinated analogues of the precursor molecules [9]. It is reported that chlorinated organic molecule decomposition products are quite stable, and can therefore provide long-lived chemical markers of chlorine exposure [12]. In the pulp and paper industry, chlorine was used for decades as the major agent to bleach pulp in order to produce white paper products. However, the extensive use of elemental chlorine for this purpose was ceased when the methods were found to produce carcinogenic chlorinated chemicals such as dioxins and other persistent organic pollutants. Chlorine's high chlorinating reactivity towards aromatic organic chemicals by an electrophilic aromatic substitution (EAS) mechanism explained the formation of the pollutants. Furthermore, it was found that alternative bleaching agents as chlorine-dioxide reduced the production of unwanted chlorinated pollutants, and today the use of chlorine in the pulp and paper industry has ceased.

Many environmental methods have been developed to identify chlorine markers such as chlorobenzenes and chlorophenols in soil [13–15], water [16] and plant materials [14]. Various methods have been used to screen these markers in the environment. For example, ultrahigh performance liquid chromatography-mass spectrometry (UHPLC-MS) was used to analyse chlorophenols in soil samples [17], gas chromatography-mass spectrometry (GC-MS) was used to determine the levels of chlorobenzenes [15] and chlorophenols [18] in water and soils, and a GC-flame ionization detector (GC-FID) was used to detect phenols and chlorophenols in the sediment [19]. Absorption spectrophotometry, iodometry and electrochemical techniques namely amperometry and voltammetry are other methods for detecting chlorine residuals [16]. Ion chromatography (IC) was also used to measure chloride in soil [13].

Chlorinated phenols were suggested as markers for "reactive chlorine" by the OPCW Fact Finding Mission on the alleged use of chlorine as a chemical weapon in Douma, in April 2018 [6]. The chlorinated phenols were identified in extracts from environmental samples such as concrete debris, collected at the scene.

Concrete is produced from ballast (sand and gravel, >80% by weight), a binder such as cement, other additives and water (for hardening), however its exact composition may vary between countries [20–22].

Six concrete samples of various ages and from various origins across Northern Europe, Middle Europe and the Middle East were selected for our chlorination studies. Various chlorination agents namely bleach, chlorine gas generated by chemical reaction, 500 ppm and neat chlorine gas were used to chlorinate concrete samples following optimised sample preparation protocols. Concrete sample extracts were analysed using targeted screening with LC-MS and GC-MS instruments before and after chlorine exposure. The same extracts were also analysed by non-targeted screening using high-resolution mass spectrometry (GC-HRMS and LC-HRMS) to look for other chlorinated organic compounds that may have formed. In addition, chlorination experiments were also performed on two different concrete samples spiked with suspected precursor material (phenol and lignin).

This study aims to examine the formation of chlorinated organic chemicals, including chlorinated phenols in concrete samples as suggested in previous report [6], with an emphasis on investigating the potential precursor molecules such as lignin or phenols for the chlorinated products. We also wanted to see if various origins and ages of concrete affect the formation of chlorinated compounds, and if possible, distinguish between an exposure to chlorine gas or another chlorinating agent such as bleach. Ultimately, this study will contribute to future research by identifying markers other than chlorophenols and chlorobenzenes, which is crucial and can assist enforcement agencies in dealing with alleged use of chlorine gas attacks.

2. Experimental design

Method development and comparison of data was done in three laboratories: VERIFIN (Finland), TNO (The Netherlands), and FOI (Sweden).

2.1. Chemicals and reagents

The following chlorinated aromatic chemicals were used: 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4-DCB), 2,4- dichlorophenol (2,4-DCP), 2,6-dichlorophenol (2,6-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP). Hexachlorobenzene (HCB) was used as an internal standard (ISTD) for GC-MS analysis, but no ISTD was used for LC-MS analysis. All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) with purity higher than 98%; details are listed in Supplementary Materials (Table S1). The stock solutions were prepared at a concentration of 10 mg/mL by dissolving the chemicals in hexane, and stored at -20 °C. The concentration of the stock solutions of these chemicals ranged from 74% to 87% was verified by GC-MS before further dilution to the working concentrations. The working solutions at the appropriate concentrations were prepared by diluting the stock solutions with hexane or ethyl acetate (EtOAc) for GC-MS analysis and methanol (MeOH) for LC-MS analysis. The working solution for GC-MS analysis contained 82 ng/mL HCB.

Lignin alkali kraft was purchased from Sigma Aldrich (Schnelldorf, Germany), and EtOAc was obtained from VWR Chemicals BDH® (Leuven, Belgium). Ammonium acetate, tert-methyl butyl ether (MTBE), hexane, MeOH, acetone and acetonitrile (ACN) were purchased from (Honeywell Fluka, Loughborough, LE, UK). LC-MS grade formic acid (98–100%) and a polytetrafluoroethylene (PTFE) syringe filter of 0.45 μm were purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) was purchased from J.T. Baker (Deventer, Holland). All reagents used were of analytical grade with purity higher than 98%. Water was purified using a Direct-Q3 UV system from Millipore (Darmstadt, Germany). The purity of helium and nitrogen gas was more than 99.995% (Woikoski, Finland). The chlorination agents used in this study include a commercial cleaning product that contains minimum 4% active chlorine (bleach), and chlorine gas at different concentrations generated by chemical reaction (TNO), 500 ppm concentration from Messer, Austria GMBH (Herzogenburg, Austria) and neat chlorine gas with purity $\geq$ 99.8% from Sigma Aldrich (Darmstadt, Germany).

Six concrete samples obtained from various sources were used in these experiments (Table 1). Concrete A, B and C were made from concrete powder containing cement and ballast produced from crushed rock material (i.e., macadam, sharped edged gravel). Concrete A was in the form of concrete powder; the sample was solidified by mixing 500 grams of concrete powder with 50 mL of tap water. Concrete B and C were both solid concrete. Concrete B contains crushed rock with sharp edges, whereas Concrete C contains natural and rounded gravel. Concrete D, E and F were solid concrete, produced in earlier decades (1930s, 1950s, and 1970s). Concretes D and E were produced from natural, rounded gravel, while Concrete F contained sharp-edged gravel, presumably from crushed rock material.

2.2. Concrete precursor spiking experiments

To study the sources of phenolic precursors in the concrete samples, two suspected precursor molecules (phenol and lignin) were used to spike the concrete samples. Lignin is a complex phenolic polymer produced in lignified wood from plants. For the phenol spiking experiment, the concrete samples were spiked with 20 μg/g of phenol in acetone. The concrete samples were then allowed to dry at room temperature before sample preparation. For the lignin experiment, approximately 0.2 or 1% of lignin was used to spike Concrete A.

Table 1
The origins and year of production of concrete samples used in this study.

Concretes	Origin	Year of production	Ballast type
Concrete A	Northern Europe	2020s	Crushed rock
Concrete B	Middle Europe	2020s	Crushed rock
Concrete C	Middle East	2020s	Natural gravel
Concrete D	Northern Europe	1930s	Natural gravel
Concrete E	Northern Europe	1950s	Natural gravel
Concrete F	Northern Europe	1970s	Crushed rock

2.3. Method development and sample preparation

All concrete samples (Concrete A, B, C, D, E and F) were ground or pulverised prior to each experiment. Each of the untreated and treated concrete samples were weighed and extracted according to procedures described in Table 2. For organic extractions, solvents containing 82 ng/mL of HCB were added to the samples before the extraction and put into a shaker or rotator for 30 minutes. The samples were centrifuged for 3 minutes at 2000 G and filtered through a 0.45 μm PTFE filter. The extract was then transferred into a screw-capped vial or a centrifuge tube. The procedure was repeated twice, and the extracts were then combined. Next, the extract was concentrated to a final volume in a mild stream of nitrogen gas at room temperature and transferred to a GC vial for GC-MS or GC-MS/MS analysis. A similar procedure was used for water and acidic water extractions, but using water and 1 M formic acid, respectively, as extraction solvents. These water and acidic water extracts were concentrated to 1 mL in a mild stream of nitrogen gas at room temperature and transferred to a vial for LC-MS or LC-MS/MS analysis. Next, 0.5 mL of the concentrated water extract was re-extracted with 0.25 mL EtOAc and analysed with GC-HRMS.

Experiments 1 to 4 were conducted to study the efficiency of extraction solvents. Experiment 5 is the Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament (ROP) [23] method, which includes organic, water and acidic water extractions. Concrete D, E and F were extracted only based on Experiment 5.

To study the recoveries of chlorinated chemicals formed in the concrete sample after chlorination, blank Concrete A (in duplicate) was spiked with the chemicals listed in Supplementary Materials (Table S1) at a final concentration of 80 μg/mL. The impact of temperature during sample preparation was examined by spiking blank Concrete A (in duplicate) with bleach and the chemicals listed in Supplementary Materials (Table S1) before drying at two different temperatures: 40 °C and room temperature. Solvent efficiency was studied by spiking bleach on three concrete samples (Concrete A, B and C) spiked with 20 μg/g of phenol prior to chlorination.

For comparative studies, one laboratory investigated the extraction solvent efficiency using blank Concrete B and C spiked with 1 μg/g of 2,4-DCP and 2,4,6-TCP prior to extraction by ultrasonic bath or agitation by rotator/shaker. They also exposed two blank concrete samples (Concrete B and C) to chlorine gas produced by chemical reaction and

Table 2
Sample preparation for untreated and treated concrete samples.

Experiment	1	2	3	4	5 (ROP method)	
Extraction method	Organic	Organic	Organic	Organic	Organic	Water or acidic water
Extracting Solvent	Hexane	EtOAc	MTBE	MTBE: ETOAc	EtOAc	Water or 1 M formic acid
Sample weight	100 mg	100 mg	100 mg	100 mg	5 g, 1 g	5 g, 1 g
Solvent volume	1 mL	1 mL	1 mL	1 mL	10 mL, 2 mL	10 mL, 2 mL
Final volume (after sample treatment)	1 mL	1 mL	1 mL	1 mL	2 mL, 0.1 mL	1 mL
Instruments	GC-MS, GC-HRMS				GC-MS, GC-HRMS	LC-MS, LC-HRMS

bleach, and extracted according to Experiment 2 (Table 2) to compare the chlorinated chemicals formed in each treatment.

2.4. Chlorination of concrete samples with various sources of reactive chlorine

The chlorination experiments for concrete samples are outlined in Table 3. The concrete samples that were treated with bleach were dried at room temperature approximately 2 hours before being transferred to an individual capped glass bottle. The pH values of all concrete samples and lignin were measured before and after chlorination (see Supplementary Materials, Table S2).

2.5. GC-MS and GC-MS/MS analysis

VERIFIN determined the GC analysis with an Agilent 7890 A gas chromatograph coupled to an Agilent 7010 GC/MS Triple Quadrupole mass spectrometer (Agilent Technologies, Wilmington, USA). The GC column was a DB-5MS UI (30 m length × 250 μm i.d × 0.25 μm film thickness) from Agilent. An Agilent 7693 autosampler was used to inject 1 μL of liquid sample in splitless mode. Helium was used as a carrier gas in constant flow mode at a rate of 1 mL/min. The oven temperature was programmed from the initial temperature of 60 °C (1 min) to reach 150 °C at the rate of 5 °C/min; thereafter, the rate was increased to 10 °C/min until the final temperature of 290 °C was reached. The MS was operated in electron ionization (EI) mode at 70 eV ionization energy. Source temperature was set at 230 °C. Nitrogen was used as collision gas at a flow rate of 1.5 mL/min, and helium quench gas was added to the collision chamber at a rate of 2.25 mL/min. The run time was 33 minutes. MassHunter (version 10.1, Agilent) was used for data acquisition and quantitative analysis. The acquisition was performed in full scan and multiple reaction monitoring (MRM) modes. In full scan mode, the scan ranged from m/z 40 to 500 with a 300 ms scan time using MS1. The chemical transitions and their collision energies are presented in Supplementary Materials (Table S3). Compounds were identified by comparing their mass spectra with reference chemicals in the NIST/EPA/NIH Mass Spectral Library (NIST 17, Gaithersburg, US) and NIST Mass Spectral Search Program (Version 2.3). The detection limits of chlorophenols by GC-MS/MS analysis are presented in Supplementary Materials (Table S4). The data was normalised by total area normalisation.

TNO used a Thermo Scientific Trace GC Ultra coupled to Thermo TSQ Quantum Ultra to perform GC analysis. A GC column VF-5MS (30 m × 0.25 mm × 0.25 μm) from Agilent was used for separation. 1 μL of liquid sample was introduced by splitless injection at 250 °C. The carrier gas (helium) was used with flow mode at a rate of 1 mL/min. The initial temperature program started at 40 °C (1 min) and was increased to the final temperature of 280 °C (4 min) at the rate of 10 °C/min. EI was carried out at 70 eV with a scan range of 0.1 s and a mass resolution of 1.0. Argon was used as a collision gas, and the selected monitoring (SRM) method with two transitions was utilised for each of the chemicals listed in Supplementary Materials (Table S1).

Table 3
Chlorination experiments.

Various sources of reactive chlorine	Organic extraction (Table 2, Experiments 1–4)	ROP method (Table 2, Experiment 5)
Bleach (VERIFIN and TNO)	0.2 mL	2 mL
Chlorine gas produced by chemical reaction (TNO)	Chlorine gas is generated by slowly dropping 20 mL of sodium hypochlorite (60–185 g active chlorine/L) into 20 mL of concentrated hydrochloric acid (37 w%). Concrete samples were exposed for 2 hours.	
500 ppm chlorine gas (VERIFIN)	Concrete samples were exposed for 1 hour with a chlorine gas flow of approximately 3 to 5 L/min. After the flow was stopped, the sample was kept in the gas chamber for another hour.	
Neat chlorine gas (VERIFIN and FOI)	Concrete samples were exposed for 1 hour with a chlorine gas flow of approximately 50 to 60 mL/min. The sample lid was closed after 1 hour of exposure and kept closed for 1 hour.	

2.6. GC-HR/MS analysis

VERIFIN used a Thermo Scientific GC Trace 1310 (Rodamo, Milan) gas chromatograph coupled to the Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer (Bremen, Germany) using the same GC column as above. A liquid sample (1 μL) was injected by a Thermo Scientific TriPlus RSH Autosampler (Zwingen, Switzerland) in a splitless injection mode. Helium gas (purity > 99.995%) was used as a carrier gas. The initial temperature program started at 40 °C and was increased to 150 °C at 5 °C/min; the final temperature (290 °C) was reached by further heating at a rate of 10 °C/min (4 min). The analysis time was 41 minutes. The source temperature was set to 230 °C, and EI was carried out at 70 eV. The transfer line was kept at 200 °C. Accurate mass measurements (HRMS) were done in the mass range of m/z 40 to 500, with a resolving power of 60,000. Internal mass calibration was conducted while measuring background ions from column bleeding as lock mass ions using (m/z) ($C_3H_9Si^+$, 73.04680; $C_3H_9O_2Si_2^+$, 133.01356; $C_5H_{15}O_3Si_3^+$, 207.03235; $C_7H_{21}O_4Si_4^+$, 281.05114; $C_9H_{27}O_5Si^+$, 355.06993). Xcalibur version 4.4 and Compound Discoverer version 3.3 (Thermo Scientific, San Jose, USA) were used for data acquisition and peak identification. Spectra obtained were compared to the NIST/EPA/NIH Mass Spectral Library (NIST 17) using NIST Mass Spectral Search Program (Version 2.3). The GC-HRMS workflow was started with the spectral selector before GC EI deconvolution. GC EI deconvolution included chromatographic peak alignment to compensate for minor differences in the retention times of the compounds, and compound detection across the input files for analysis. NIST Libraries were selected for the Search Libraries parameter which was used to identify the detected compounds. This workflow also imputes areas for missing chromatographic peaks. Molecular networks were created before adding descriptive statistics, and differential analysis was performed to visualise compounds that may be related. The chromatographic data was normalised by total area normalisation and filtered by extracting the peaks corresponding to analytes containing only chlorine isotopes. Only chlorinated organic compounds with a High Resolution Filtering (HRF) value higher than 80% were selected for identification of compounds in the sample. Thereafter, the data was analysed by principal component analysis (PCA), or orthogonal partial least squares discriminant analysis (OPLS-DA) performed in SIMCA (version 17, Sartorius, Stedim Biotech). Elemental compositions, theoretical and measured masses and mass error were also calculated and confirmed for each chemical. The OPCW criterion for mass error tolerance in the Environmental Proficiency Tests states that the mass error for the reported chemicals should be less than 5 parts per million (ppm) [24].

FOI coupled the same GC system as VERIFIN to an Exactive orbitrap mass spectrometer. The injector was set at 200 °C and 1 μL of the sample was splitless injected. An Agilent DB-5ms column (30 m length, 250 μm i.d × 0.25 μm film thickness) was used. The temperature program started at 40 °C (1 min) and ramped at 10 °C/min until 300 °C (hold 5 min), with a total runtime of 32 minutes. Helium was used as a carrier gas and the flow rate was 1.2 mL/min. The ion source was set at 230 °C, transfer line at 250 °C, EI was 70 eV, mass range was m/z 30 to 550, and the resolution was 30,000. Lock masses (207.03235, 225.04292, and 281.05114) were used for internal calibration. The instrument was tuned and calibrated daily. The data was processed by Xcalibur (version 4.2) and Compound Discoverer (version 3.2).

2.7. UHPLC-MS and UHPLC-MS/MS analysis

The analysis at VERIFIN was performed by UHPLC-MS using a Waters Acquity UPLC I-Class instrument from Waters Corporation (Milford, MA, USA) coupled to a Waters Xevo TQ-D triple quadrupole mass

spectrometer (MS/MS) from Waters Corporation (Milford, MA, USA). 2 mM of ammonium acetate (A) and ACN (B) were used as the mobile phases for the separation of the analytes on a Waters XBridge BEH C18 (2.1 × 100 mm, 2.5 μm) column at 25 °C. The flow rate was set at 0.5 mL/min; initial conditions were 5% B, which then gradually increased to 100% B in 3.5 min and held for 0.5 min. Next, the B ratio was reduced to 5% B within 0.5 min, and the column was then equilibrated for 1 min at 5% B. The injection volume was 5 μL. The acquisition was performed in full scan and MRM modes after both positive and negative electrospray ionization (ESI) with nitrogen (N_2) as the spray gas and argon (Ar_2) as the collision gas. The chemical transitions and their collision energies are presented in Supplementary Materials (Table S5). The mass range from *m/z* 60 to 600 was used for full scan screening. The instrument parameters were set as follows: capillary voltage 0.80 kV, cone voltage 20 V, source temperature 120 °C, desolvation temperature 500 °C, desolvation gas flow of 1000 L/hr and collision gas flow of 0.15 L/min. Masslynx (version 4.1) was used for data acquisition. The data was normalised by total area normalisation before further analysis. The detection limits of chlorophenols by UHPLC-MS/MS analysis are presented in Supplementary Materials (Table S4).

2.8. UHPLC-HRMS analysis

VERIFIN utilised a Thermo Scientific Ultimate 3000 UHPLC (Germering, Germany) coupled to a Thermo Scientific Orbitrap Fusion mass spectrometer (San Jose, USA) to confirm the chemicals found and for non-targeted screening chemicals. Eluents, LC column, the chromatographic gradients and the injection volume were the same as in the LC-MS analysis. The column temperature was 25 °C. The acquisition in full scan MS mode was performed using heated electrospray ionization (HESI) in both positive and negative ion modes with nitrogen (N_2) as the spray gas. The instrument parameters were set as follows: spray voltage 3.5 kV (positive) and 2.5 kV (negative), source temperature 300 °C, ion transfer tube temperature 350 °C, sheath gas 40 Arb, auxiliary gas 15 Arb, and sweep gas 0 Arb. Accurate mass measurements (HRMS) were performed in the mass range of *m/z* 70 to 600 using an RF lens at 60% and the resolution of 120,000. Mass accuracy of the instrument was specified to be $\leq$ 5 ppm using internal calibration. Xcalibur (version 4.2) and Compound Discoverer 3.3 (Thermo Scientific, San Jose, USA) were used for data acquisition and peak identification. For the LC-HRMS workflow, retention time alignment was performed before unknown compound detection across all peaks. Elemental compositions were predicted, and all compounds were identified using mzCloud, ChemSpider (exact mass or formula) and a local database search against Mass Lists (exact mass with or without RT). A spectral similarity search against mzCloud was performed, and mzLogic was applied to rank the structure candidates from ChemSpider and the mass list matches. Compounds were annotated before differential analysis of the detected compounds. The data was normalised by total area normalisation before further analysis with SIMCA as described in the GC-HRMS analysis.

2.9. Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS)

A Flash EA 2000 elemental analyser (Thermo Fisher Scientific, Bremen, Germany) coupled to a Delta V Isotope Ratio Mass Spectrometer was used to measure the carbon content of concrete samples. Carbon of the dried concrete samples was converted to CO_2 by combustion. Dried concrete samples were defined by oven drying at 70 °C for a minimum of 18 hours. Mass spectrometric measurement was performed on CO_2 to yield a mass fraction of 40 to 50% of C (g C per g dry mass), and the results were corrected for drift and sample size effect (non-linearity). Wheat and maize flours were used as working standards to calibrate against reference standards (cyclohexanone, nicotinamide, and sucrose).

2.10. Pyrolysis GC-MS (Py-GC-MS)

The pyrolysis GC-MS analysis was performed at the Biopolymer Analytical Platform at the Umeå Plant Science Center. An Agilent 7890A-5975C GC-MS system (Agilent, Agilent Technologies AB, Sweden) coupled to an oven pyrolyser equipped with an PY-2020iD and AS-1020E auto sampler (FrontierLabs, Japan) was used. Approximately 200 ± 10 μg of concrete samples were weighed (in triplicate) and transferred to autosampler containers, and pyrolysed at 450 °C. Helium was used as the carrier gas, and the samples were injected in a 16:1 split ratio. The pyrolysate was separated using a capillary column DB-5MS (30 m × 0.25 mm i.d., 0.25 mm film thickness) (J&W, Agilent Technologies AB, Sweden). The GC temperature program increased from 40 °C to 320 °C at a rate of 10 °C/min and was held for 3 minutes. The temperature of the GC-MS interface was kept constant at 300 °C. The mass spectrometer was equipped with a quadrupole type analyser, and scanned the mass range from *m/z* 30 to 500 at 3.1 scan/s. Ionisation was accomplished through the use of a 70 eV electron bombardment. The Py-GC interface and GC injector temperatures were set to 340 °C and 320 °C, respectively. After 1 min, the gas-saver mode with a flow rate of 3 mL/min was used to vent away the pyrolysate bleed of the remaining sample in the pyrolyser oven.

2.11. Quality assurance (QA) and quality control (QC)

The performance of the instruments was accessed using the laboratory's QC test solutions and system blank test before samples analysis. System blank, solvent used in sample preparation to demonstrate the absence of the tested chemicals in the reagents, solvents, or equipment used prior to sample analysis. Separate QC solutions are used for laboratories' GC and LC measurements. Samples were analysed after all these tests had been accepted. The column performance test was performed in each sample batch prior to sample analysis, between sample analysis at least every 20th sample, and after sample analysis. The GC and LC system criteria were met if each test compound or identified compounds gave a peak with a signal-to-noise more than 5 in the total ion chromatograms (TICs) or extracted ion chromatograms (EICs) [23]. Chlorinated compounds were identified by comparing the measured spectra of the identified compounds to those in the NIST libraries, with a match factor of at least 80% [24]. For GC-HRMS and LC-HRMS data, the mass accuracy with a mass error < 5 ppm must be meet [24].

3. Results and discussion

3.1. Method development

For method development, four different organic extraction solvents were studied: hexane, EtOAc, MTBE and a MTBE:EtOAc (1:1) mixture (Table 2). EtOAc was significantly better as an extraction solvent than the alternatives, and was hence chosen as the organic extraction solvent for the GC-MS analysis. For extraction of more polar organic chemicals to be analysed by LC-MS, the extraction efficacy of water and acidic water was compared. This revealed that the water extraction was superior in Supplementary Materials (Fig. S1). With all tested methods, no chlorinated chemicals were found in the blank concrete samples.

Recovery studies showed that the concrete sample spiked with the chemicals in Supplementary Materials (Table S1) heated at 40 °C had significant losses, with less than 10% of chlorobenzenes detected and no chlorophenols found in concrete extracts. This recovery study was repeated by drying at different temperatures, showed that the sample dried at room temperature had almost three times higher peak area intensities than the sample dried at 40 °C. It was found that volatile chemicals like chlorophenols and chlorobenzenes evaporate at 40 °C. As such, no heating was used in the following sample preparation protocols.

The efficiency of solvent extraction was studied by using three

concrete samples (Concrete A, B and C) spiked with 20 μg/g of phenol and treated with bleach before extraction according to Experiments 1 to 4 (Table 2). These extracts were analysed for targeted chlorophenols and chlorobenzenes (Supplementary Materials, Table S1) by LC-MS/MS and GC-MS/MS in the MRM mode. Fig. 1 shows the GC-MS/MS analysis results. Dichlorobenzenes were detected with significantly lower intensities than chlorophenols, and therefore are not displayed in Fig. 1. This extraction efficiency experiment was also carried out in one laboratory by spiking 2,4-DCP and 2,4,6-TCP to blank Concrete B and C before extraction according to Experiments 1 and 2 (Table 2). The results showed that EtOAc compared to hexane extraction produced better results, with recoveries 20% and 8% (data not shown), respectively. Despite the low percentage of study extraction recovery with EtOAc, it outperforms hexane extraction. EtOAc was chosen as an extraction solvent for organic extractions because it was the most effective extracting solvent among the four studied organic solvents in both blank concrete and concrete spiked with phenol prior to chlorination.

Extractions by ultrasonic bath or agitation by rotator were studied for the extraction of Concrete B and C spiked with 1 μg/g of 2,4-DCP and 2,4,6-TCP (Table 2: Experiments 1 and 2). In parallel, the use of shaker-supported extraction was found to give comparable recoveries. The results showed that the combination of EtOAc with a rotator for one hour was superior to ultrasonic bath, with recovery of more than 98%. Both the shaker and the rotator can be used to extract samples of untreated and treated concrete samples.

Thereafter, the ROP method (Table 2, Experiment 5) using EtOAc and water extractions were selected for the preparation of concrete samples. The use of EtOAc extraction and GC-MS analysis, in combination with water extraction and LC-MS analysis enabled the chemical analysis of a broad range of chlorinated chemicals of different polarities. In addition, water-to-organic extraction procedures were carried out for the GC-MS analysis, which has lower limits for detecting chlorophenols in the extract (Supplementary Materials Table S4). It was shown that water is more effective than acidic water in extracting chlorinated organic chemicals from concrete samples (Supplementary Materials Fig. S1), but the water-to-organic extraction allows the superior GC-MS analysis to be used for detection of chlorinated compounds. In water extracts, chlorinated phenols were the major species of chlorinated chemicals detected when extracts were analysed by LC-MS in the full scan mode (data not shown).

3.2. Screening for chlorinated organic molecules in concrete exposed to reactive chlorine

The experiments above identified chlorinated phenols as major products from chlorination of concretes by 500 ppm chlorine gas. Specifically, chlorinated phenols were detected in extracts from three different concrete samples (A, B and C) following chlorination with 500 ppm chlorine gas (Supplementary Materials Fig. S1, Fig. S2 and S3). Monochlorophenol (MCP) was the main chlorophenol detected, followed by dichlorophenol (DCP) and trichlorophenol (TCP), which were present at 7 to 11% and 17 to 35% relative intensity, respectively. The levels of chlorinated phenols differed between the different concretes, with the lowest levels being found in Concrete A, as shown in Supplementary Materials (Fig. S1). The concretes A, B and C were all produced from a modern concrete powder and contained relatively low levels of chlorinated organic chemicals following exposure to 500 ppm chlorine. This data indicated that there was a difference in the content of suitable precursor molecules in the various concrete types. This trend was confirmed by studies of two old concrete samples; Concrete D and E from the 1930s and 1950s, respectively. Following chlorination by neat chlorine, these samples contained a large number of chlorinated organic chemicals, present at high levels. EA-IRMS analysis was performed on four different concrete samples (Concrete A, D, E, and F) to determine the carbon content of each sample (Supplementary Materials Table S6). The results revealed that Concrete D (1930s) and E (1950s) had higher carbon content than Concrete A and Concrete F (1970s) by a factor of nearly five. This data suggests that Concrete D and E produced more chlorinated chemicals as a result of a higher content of precursor molecules in these two concretes.

Another experiment was carried out on two blank concrete samples (Concrete B and C) that were treated with chlorine gas produced by a chemical reaction and bleach, and then extracted as described in Experiment 2 (Table 2). The results revealed that TCP was produced at higher levels than DCP by both species of reactive chlorine when EtOAc extracts were analysed by GC-MS/MS in the MRM mode (Supplementary Materials Fig. S4). This experiment demonstrated that bleach contains more reactive chlorine than chlorine gas produced by chemical reaction, as concrete samples treated with bleach contained ten times more TCP. However, the moisture level of the chlorine produced from bleach and hydrochloric acid is not known, and no drying gas was applied.

3.3. Non-targeted screening by HRMS instruments

Non-targeted screening for chlorinated organic compounds was performed using combined GC-HRMS and LC-HRMS data from the analysis of organic and water extracts, respectively. The comparison of data from untreated and treated concrete samples identified a range of chlorinated chemicals, including the chlorinated phenols discussed above.

Chlorinated organic compounds detected only in the chlorinated concrete samples are listed in Table 4, and the analytical techniques

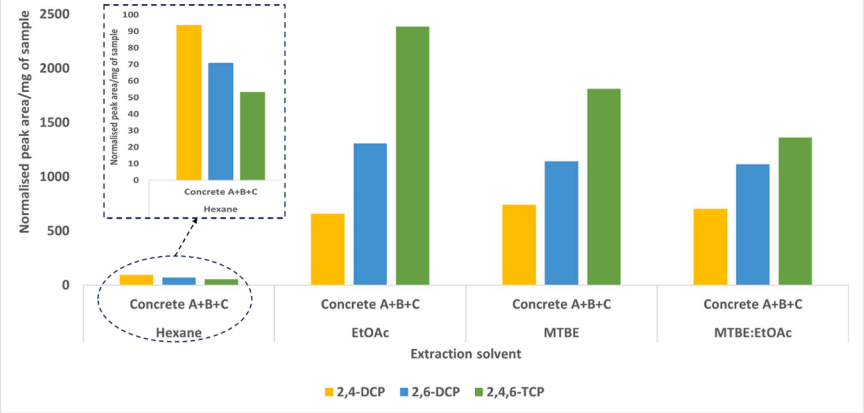

Fig. 1. Extraction efficiency of different solvents as the sum of three concrete samples (Concrete A, B and C) spiked with 20 μg/g of phenol prior to chlorination with bleach and extracted according to Table 2 (Experiments 1 to 4). Formed chlorophenols in each treated concrete sample using four extraction solvents when analysed by GC-MS/MS in the MRM mode (normalised peak areas in per mg of sample).

Table 4
Chlorinated chemicals found after chlorination when extracted according to the ROP method (Table 2, Experiment 5) and analysed using LC-HRMS and GC-HRMS.

Concrete	A	B	C	D	E	F	A+P	A+L	L	A	B	C	A+P	A+L	L	A	B	C	D	E	F	A+P	A+L	L
Chlorination method	Bleach									Cl2 (g) 500 ppm						Cl2 (g) neat								
Chlorophenol	-	-	-	-	-	-	-	x	x	x	-	-	-	x	x	x	x	x	-	-	-	-	-	x
Dichlorophenol	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Trichlorophenol	x	x	x	x	x	x	-	x	-	x	-	-	x	x	x	x	x	x	x	x	x	x	x	x
Tetrachlorophenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
Chlorophenoxyphenol	-	x	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-
Dichlorophenoxyphenol	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-
5-Chloro-2-(2,4-dichlorophenoxy)phenol	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-
Dichloromethoxyphenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	-	x	-	x	x
Trichloromethoxyphenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x
Tetrachloromethoxyphenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x
3,5-Dichloro-2,6-dimethoxyphenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-
3,5-Dichloro-4-(hydroxymethyl)phenol	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloronitrophenol	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-
Dichloronitrophenol	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-
2-Chloro-4-nonylphenol	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorobenzene	-	-	-	-	-	-	-	-	-	x	x	x	x	x	-	x	x	x	-	-	-	-	x	-
Dichlorobenzene	x	-	-	-	-	-	-	x	-	x	x	x	-	-	-	x	x	x	x	x	x	x	x	-
Chloromethylbenzene	x	x	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dichloromethylbenzene	x	x	x	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichloromethylbenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	x	x	x	x	-
Chlorodimethylbenzene	-	-	-	-	-	-	-	-	-	-	x	x	x	-	-	x	x	x	-	-	-	-	-	-
Pentachloromethoxybenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
1,4-Dichloro-2,5-dimethoxybenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Chlorophenoxybenzene	x	-	x	-	-	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dihydroxychlorobenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	x	x	x	x	-
2,3,5-Trichloro-6-hydroxy-1,4-benzoquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
Dichloroacetic acid	x	x	x	x	-	x	x	x	x	-	-	-	-	-	-	x	x	x	x	x	x	x	x	-
Trichloroacetic acid	x	x	x	x	-	x	-	x	x	-	-	-	-	-	-	x	x	x	x	x	x	x	x	-
4-Chlorophenoxyacetic acid	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	x	-	x	x	-	x	x	-
Dichlorophenoxyacetic acid (2,4-D)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	x	-	x	x	-
Trichlorophenoxyacetic acid (2,4,5-T)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
4-Chlorobenzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-	x	x	x	x	-
2,4-Dichlorobenzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	x	x	x	-
3,5-Dichloro-2-hydroxybenzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	x	x	-
5-Chloro-2,4-dihydroxybenzoic acid	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-Chloro-6-hydroxy-2-methoxybenzoic acid	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
3,6-Dichloro-5-hydroxy-2-methoxybenzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	-
2,2-Dichloropropionic acid	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-(3-Chlorophenoxy)propanoic acid	-	-	-	-	-	-	-	x	x	-	-	-	-	x	x	-	-	-	-	-	-	-	x	-
2-(4-Chloro-2-methylphenoxy)propanoic acid	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloral hydrate	-	-	-	-	-	-	-	-	-	x	x	x	x	-	-	x	x	x	x	x	x	x	x	-
2′,5′-dichloro-[1,1′-Biphenyl]− 2-ol	x	x	x	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4-Hydroxy-2′,3,5′-trichlorobiphenyl	x	x	x	x	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*X, present in the sample; -, not present in the sample; A+P, Concrete A spiked with 20 μg/g phenol prior to chlorination; A+L, Concrete A spiked with 0.2 or 1% lignin prior to chlorination; L, Lignin after chlorination.

used for their identification can be referred to in the Supplementary Materials (Table S7A and S7B). The combination of LC-HRMS and GC-HRMS analyses allowed a broad range of chemicals to be detected including non-chlorinated phenol. Detected polychlorinated chemicals include chlorinated phenols, chlorobenzenes, and chlorinated acids.

The comparison of concrete A, B and C, following chlorination by bleach and 500 ppm chlorine gas indicated some minor differences between the different species of reactive chlorine. Generally, bleach produced a larger number of chlorinated compounds as compared to 500 ppm chlorine gas (Table 4). The bleach had a reactive chlorine concentration of 40,000 ppm as compared to 500 ppm concentration of chlorine gas. It is well known that molecular chlorine has a much higher reactivity than sodium hypochlorite, which is the reactive chlorine species present in bleach [25,26]. However, the increased reactivity of chlorine gas cannot compensate for the much higher concentration of reactive chlorine in bleach (40,000 ppm) than in chlorine gas (500 ppm), respectively.

The exposure of the different concretes by neat chlorine produced a higher number of chlorinated chemicals, and many of them were only found in concrete samples exposed to neat chlorine. Two examples are trichloromethylbenzene and tetrachlorophenol (TeCP), which are unique markers to the exposure with neat chlorine gas. Trichloromethylbenzene was identified in all different exposed concretes, while TeCP was only detected in the old concretes (D and E) with a higher carbon content than the other concretes shown in Supplementary Material (Table S6). Concrete D (1930s) also contained chloromethoxyphenols (chloroguaicols) and chlorodimethoxyphenols (chlorosyringols). Chloronitrophenols and chlorophenoxyphenols were discovered in both Concrete B (Northern Europe) and C (Middle East) treated with 500 ppm chlorine gas. Chloral hydrate was found when concrete samples were treated with only chlorine gas (500 ppm and neat).

Different chlorobenzenes were also observed, such as chloromethylbenzenes and chloromethoxybenzene when EtOAc extracts of treated concrete samples were analysed with the GC-HRMS instrument. Benzyl chloride or chloromethylbenzene was only detected in modern concrete, namely Concrete A, B, and C, which were produced using modern technology, treated with bleach, and analysed by GC-HRMS. The molecule structures of these chlorinated compounds can be referred to the Supplementary Materials (Table S8).

Chlorinated phenols and chlorobenzenes are classes of chemicals with an anthropogenic origin (i.e., are not produced by any natural processes). The main industrial method for TCP production is the reaction of phenol with chlorine gas, but phenol can also be chlorinated to chlorophenols with sodium hypochlorite, the main component of chlorine-based bleach [27]. However, the production of highly chlorinated organic compounds such as TeCP requires higher reactivity of chlorine gas. The industrial production of the pesticide pentachlorophenol requires the use of concentrated chlorine gas, a high reaction temperature and the presence of a catalyst [28,29]. This process produces pentachlorophenol at a yield of up to 90% and 5 to 10% TeCP as a by-product.

The detected chloroacetic acids in the chlorine-exposed concretes are not specific to chlorine gas and have been reported as chemicals present in soil as a natural process of chlorination of organic compounds. Other compounds specific to exposure to neat chlorine are chlorinated acids such as chlorophenoxyacetic and chlorobenzoic acids.

To visualise the systematic difference between untreated and treated concrete samples, LC-HRMS data were analyzed by multivariate data analysis using PCA and OPLS-DA to compare data from treated and untreated concrete samples. The data from untreated concrete samples only contains background noise (instrument and chemical noise). The PCA score plot showed a clustering according to the species of chlorination agent used (data not shown). Using supervised OPLS-DA, the data was classified according to their chlorination treatment method, including untreated, bleach, and chlorine gas (500 ppm or neat). The OPLS-DA score plot for LC-HRMS data displayed a distinct separation between bleach as a chlorinating agent and untreated and treated (chlorine gas at different concentrations) concretes. All four classes (untreated and three chlorination method) were separated in a model consisting of four predictive components as shows in Fig. 2. The separation of samples by the OPLS-DA model, was further displayed in the dendrogram plot for LC-HRMS in Fig. 3.

The OPLS-DA multivariate data analysis was also repeated for the GC-HRMS data. The largest separation in data (1st component) describes the difference between neat chlorine and the other samples (Supplementary Materials Fig. S5). The second component separates the bleach samples from the 500 ppm chlorine gas and the untreated concretes. The dendrogram plot for GC-HRMS data is also shown in the Supplementary Materials (Fig. S5). Neat chlorine gas produces a large number of chlorinated chemicals, present at relative high concentrations, giving rise to a clear separation from the other classes in the model. Concrete samples exposed to bleach, separate from a mixed cluster of untreated and 500 ppm chlorine gas treated concrete samples. The low concentration of 500 ppm chlorine gas gave rise to a low number of chlorinated chemicals at low concentration resulting in a poor separation to the untreated samples. As discussed above, the higher reactivity of chlorine gas (at 500 ppm) cannot compensate for the very big concentration difference to 40,000 ppm reactive chlorine in bleach.

The explanation for the different appearances of the OPLS-DA score plots is the data they are built on. In comparison to the GC-HRMS analysis of the EtOAc extract, the LC-HRMS analysis of the water extract will, in part, detect different chemicals. However, a clustering of the samples based on the species of reactive chlorine used can be observed.

3.4. Sources of phenolic precursors in concrete samples

The correlation between the carbon content of different concretes and the level of chlorinated chemicals following exposure to reactive chlorine species motivated a deeper investigation of the potential precursor molecules present in the concretes. Since chlorinated phenols were the most abundant chlorinated compounds found in exposed concretes, the obvious potential precursor molecule to consider is phenol, although it is known that simple phenols, pyrocatechol, hydroquinone, and resorcinol are less common in nature [30]. In extracts from blank concretes, low levels of phenol were detected (data not shown) and spiking experiments were performed to assess the importance of phenol as a precursor of highly chlorinated phenols [31,32].

Samples of concrete A or B were spiked with 20 μg/g phenol prior to chlorination with bleach and chlorine at both 500 ppm and neat concentration. The analytical data demonstrated no formation of highly chlorinated chemicals, matching the data of concrete D and E (Table 4). The increased levels of DCP and TCP in Concrete B after the exposure to bleach are shown in Supplementary Materials (Fig. S6). This is in line with reports of phenol being chlorinated to produce chlorophenols by sodium hypochlorite, the main component of chlorine-based bleach [27]. However, the data indicates that phenol is not a precursor of highly chlorinated compounds such as TeCP or pentachloromethoxybenzene.

An alternative precursor for the formation of chlorinated phenols is lignin, the phenolic polymer present in wood of all lignified plants. Guaiacol and syringol are derived from the pyrolysis of lignin and have been shown to be chlorinated to chloroguaiacols, and chlorosyringols when exposed to high concentrations of chlorine [27,33]. The formation of highly chlorinated organic molecules such as TeCP, chlorinated dibenzofurans, and dioxins was a significant problem when the pulp and paper industry used chlorine to bleach paper pulp for the removal of residual lignin in the pulp, thereby whitening the paper products [33,34]. This supports the hypothesis that lignin is a potential precursor for such chemicals when concretes with a lignin content are exposed to chlorine gas.

Litter from dead plants will be embedded in the soils and become part of the soil organic matter. Lignin is relatively difficult for

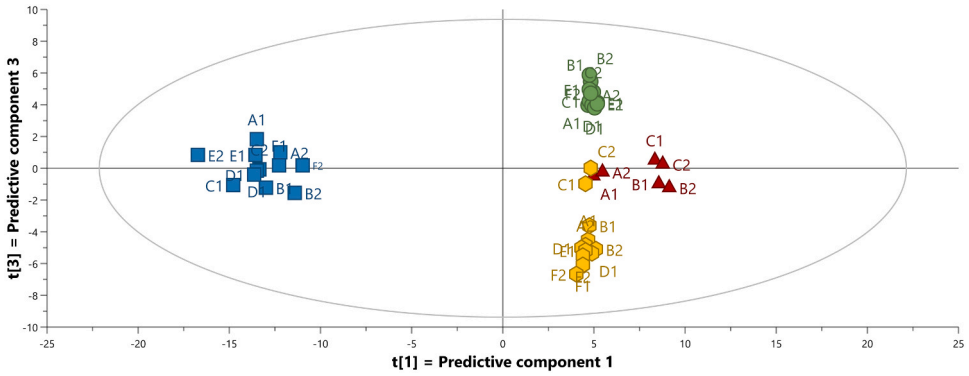

Fig. 2. OPLS-DA score plot for LC-HRMS data generated by SIMCA version 17 for six concrete samples treated with different chlorination (duplicate samples). The data was put into classes according to their chlorination treatment method. The model consists of two predictive components: t[1]= scores for predictive component 1, t[3]= scores for predictive component 3. (R_2X_{cum}= 0.469; Q^2_{cum}= 0.87). Untreated (●), bleach (■), 500 ppm chlorine gas (▲), and neat chlorine gas (●).

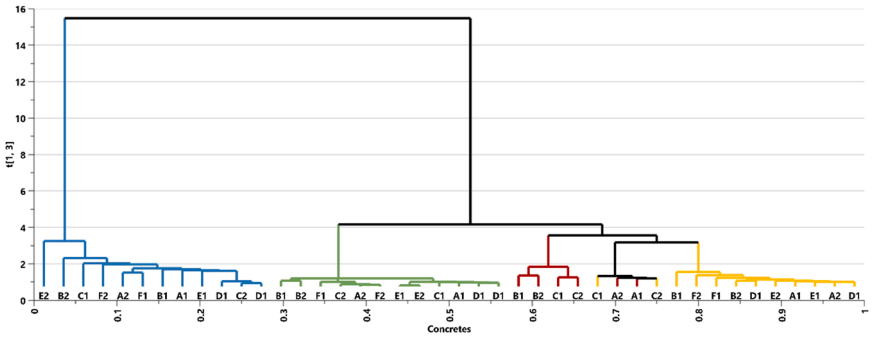

Fig. 3. Dendrogram plot for LC-HRMS data generated by SIMCA version 17 for six concrete samples treated with different chlorination (duplicate samples). The data was put into classes based on their chlorination treatment method and connected using a single linkage. The model consists of two predictive components: t[1]= scores for predictive component 1, t[3]= scores for predictive component 3. Untreated (●), bleach (■), 500 ppm chlorine gas (▲), and neat chlorine gas (▲).

microorganisms to degrade and will control the litter decomposition and production of dissolved organic matter [35]. The relative enrichment of aromatic compounds in dissolved organic matter in soil further supports the hypothesis that compounds like lignin monomers are stable in the soils [31,32]. Our hypothesis is that partially degraded lignin will be present in concrete ballast derived from surface pits. The lignin will provide a source of phenolic precursors to produce chlorinated phenols after chlorine exposure. When Concrete A spiked with alkaline lignin, was chlorinated with neat chlorine gas, a dramatic increase of highly chlorinated phenols and benzenes was observed (Fig. 4). Highly chlorinated compounds such as TeCP and pentachloromethoxybenzene were detected in this concrete sample spiked with lignin after chlorination (Table 4). These chemicals were not produced by either the exposure of bleach or 500 ppm chlorine. Fig. 4 does not show the chlorinated chemicals exposed to 500 ppm chlorine gas, since it produced the least chlorinated chemicals.

The formation of chloroguaiacols, chlorosyringols and chlorocatechol demonstrated the presence of phenolic precursors of lignin in

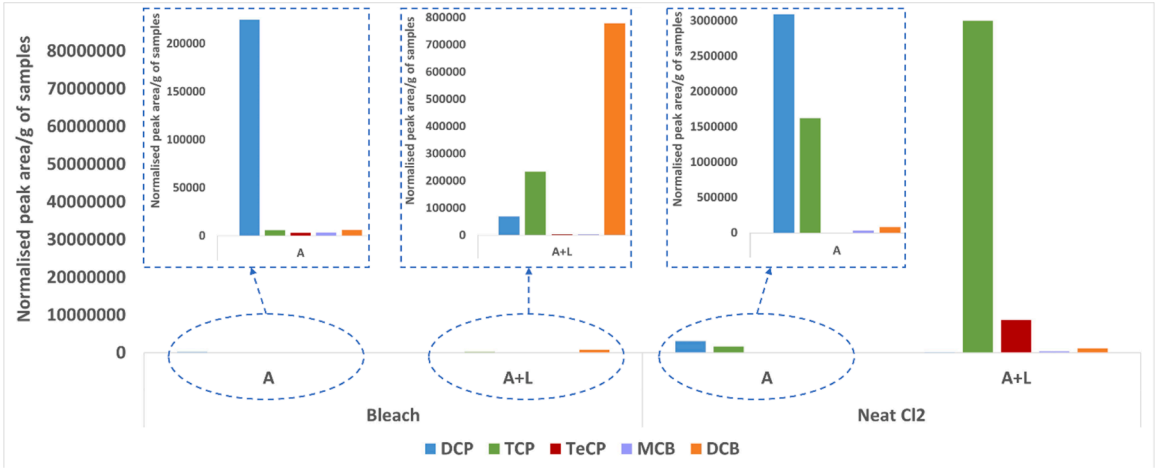

Fig. 4. Results of EtOAc extracts of chlorinated concrete samples (Concrete A without (A) and spiked with 1% of lignin (A+L)) and analysed using GC-HRMS (duplicate samples). These concrete samples were chlorinated with bleach and neat chlorine gas and extracted according to the ROP method (Table 2, Experiment 5) (normalised peak areas in per g of sample).

concrete samples, which was supported by the pyrolysis GC-MS experiment. As these highly chlorinated compounds were also detected in Concrete D (1930s) and Concrete E (1950s) after chlorination with neat chlorine gas, the question arises whether lignin is present in these old concretes. The analysis of carbon content by EA-IRMS indicated that the Concrete E had a five-fold higher level than Concrete A (Supplementary Materials Table S6). This correlation data suggests that Concrete D and E produced more chlorinated chemicals as a result of a higher content of precursor molecules in these two concretes, but the EA-IRMS technique does not provide any information on the identity of the carbon containing chemicals. The carbon content does not provide any such information but the correlation between carbon content and production of chlorinated phenols. Further analysis of Concrete E by pyrolysis-GC-MS provided evidence for the presence of lignin or degraded lignin (i.e. monolignols) in the samples by the detection of the lignin pyrolysis products (Supplementary Material Fig. S7). This provides support for the hypothesis of lignin being an important precursor of chlorinated compounds in this concrete type.

The old Concrete D and E contained rounded gravel stones, indicating that they are produced from natural sand and gravel collected in pits. On the other hand, the modern concretes contain sharp-edged gravel, indicating that they are produced from crushed rock or stone material. This type of ballast is preferred, since the sharp edges increase the mechanical strength of the hardened concrete. Rock material used for production of crushed ballast can be expected to only contain very low levels of lignin. In addition, the crushed ballast material is normally washed by water to remove ground fine material, thereby further reducing the content of any lignin in the ballast. Therefore, modern concrete produced by using ballast from crushed rock may also contain sufficient amounts of lignin-derived chemicals to allow for the production of chlorinated chemicals upon exposure to chlorine gas. Moreover, lignins are also used as a vital “fourth” ingredient of concrete as chemical additive/admixtures [36]. When lignosulphonate (commercial lignin by-product) is added to a cement-water system, it acts as an adhesive binder and can improve concrete workability by acting as a water-reducing agent, pumpability aid, and integral water-proofer [37].

3.5. Chlorinated organic molecules in concrete as markers for chlorine exposure

This study indicates that concretes differ in their content of organic compounds as potential precursors to produce their chlorinated analogues after exposure to reactive chlorine. Chlorination has significantly impacted old concrete samples, especially those from the 1930s (Concrete D) and 1950s (Concrete E), followed by samples from Middle Europe (Concrete B), the Middle East (Concrete C), Northern Europe (Concrete A) and an old concrete sample from the 1970s (Concrete F).

Chlorination of modern concretes indicates that they contain a low level of these precursors, independent of their geographical origin. In old concretes produced by the use of ballasts from natural sand and gravel pits, lignin is commonly present as a precursor molecule. This implies that different chemical markers for chlorine exposure must be considered due to the heterogeneity of concretes. The chlorinated organic compound trichloromethylbenzene is of interest, since it is present in all investigated concretes and is chlorinated by neat chlorine gas. In lignin containing concretes, TeCP and pentachloromethoxybenzene are examples of significant markers following chlorination.

Another observation is the shift to higher chlorinated compounds when the concentration of a chlorinating agent is increased (Supplementary Materials Fig. S6). When the bleach concentration ranged from 10% to 50%, 2,6-DCP showed the highest amount, and when the concrete sample was exposed to undiluted bleach (100%), 2,4,6-TCP had the highest relative intensity. This clarified that the chlorophenol species formed were proportional to the exposure level of reactive chlorine and level of phenolic precursors in the samples [38]. The shift to higher chlorinated compounds could be explained by a depleted precursor pool following high exposure levels. The tendency for a reduced total pool of chlorinated phenols observed in the experiments with undiluted bleach may be explained by the reported ring cleavage of chlorinated phenols by high hypochlorite doses in aqueous solutions with high pH [39,40].

4. Conclusions

This study identified chlorinated compounds as specific markers for the exposure of concrete material to bleach, and 500 ppm and neat chlorine gas. Overall, different levels and species of chlorinated organic compounds namely chlorophenols, chlorobenzenes, and chloromethylbenzenes were identified. There was a clear difference in patterns of formed chlorinated compounds and therefore exposure to bleach and chlorine gas can possibly be differentiated e.g., chloral hydrate was identified only from concrete debris after chlorine gas exposure. Two other examples of diagnostic markers for neat chlorine exposure were trichloromethylbenzene and tetrachlorophenol produced in all concrete samples. Multivariate data analysis (OPLS-DA) for concrete treated with various chlorination shows a distinct separation of bleach and chlorine gas at different concentrations.

By studying six concrete materials of different origin, the formation of chlorinated compounds by different species of reactive chlorine were monitored. A range of anthropogenic chlorinated compounds were discovered such as chlorophenols, chlorobenzenes, and chloromethylbenzenes, and their abundance was found to differ greatly between different concrete materials. Among the six different concrete samples, an old sample from the 1950s (Concrete E) had the highest levels of chlorinated chemicals, including TeCP, after chlorination. In this concrete material, the presence of lignin was detected by lignin markers in pyrolysis-GC-MS. To study the dependence of chlorinated chemical formation of suitable precursors present in the concrete samples, Concrete A was spiked with phenol and lignin prior to chlorination. TCP was the most prevalent chlorinated chemical found in concrete samples spiked with phenol. However, when Concrete A was spiked with lignin prior to chlorination by neat chlorine, TeCP was found together with a number of neat chorine-specific chlorinated compounds. This finding suggests that old buildings may have included lignin in their concrete compositions. From our study, it improves knowledge that the chlorinated compounds formed during chlorination depend on the concentrations of reactive chlorine and the precursors present in the concrete. Lignin has been proposed as one of the phenolic precursors, while the other precursors are largely unknown and may differ depending on origin and age of analysed concrete samples. The chlorinated chemicals and specific markers discovered in our research assist laboratories in forensic investigations of alleged use of chlorine gas attacks.

Environmental implication

This study examines six concrete samples of various ages and from various origins that were exposed to various sources of reactive chlorine including commercial bleach and chlorine gas with different concentrations. Chlorine has recently been used as a chemical weapon in the Syrian Arab Republic and Iraq. Higher concentrations of chlorine gas exposure result in death and produces carcinogenic chlorinated chemicals such as dioxins and other persistent organic pollutants. We listed the chlorinated chemicals discovered in our research and identified specific markers for chlorine gas, so that other laboratories may use them in forensic investigations of chlorine attacks.

CRediT authorship contribution statement

Nurhazlina Hamzah: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Karin Höjer Holmgren:** Validation, Formal analysis, Investigation, Writing - review

& editing. **Crister Åstot:** Conceptualization, Resources, Writing - review & editing. **Marcel J. van der Schans:** Investigation and Validation. **Leo de Reuver:** Investigation and Validation. **Paula Vanninen:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgements

This work at VERIFIN was supported by the Ministry of Foreign Affairs, Finland and the Public Service Department, Government of Malaysia. The work at FOI was supported by the Ministry of Defense, Sweden. The authors also acknowledge Junko Takahashi Schmidt at the Biopolymer Analytical Platform at Umeå Plant Science Center for her valuable support with pyrolysis-GC-MS. The NTA Study Reporting Tool (SRT) was used during peer review to document and improve the reporting and transparency of this study. (10.1021/acs.analchem.1c02621; 10.6084/m9.figshare.19763482 [PDF] or 10.6084/m9.figshare.19763503 [Excel]). Funding for open access was provided by the Helsinki University Library.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132332.

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