Forensic Chemistry 45 (2025) 100680

Contents lists available at ScienceDirect

Forensic Chemistry

journal homepage: www.sciencedirect.com/journal/forensic-chemistry

Discovery of selective markers for chlorine gas exposure in concrete

Mirjam de Bruin-Hoegée [a,*], Bence Dallos [b], Tomas van Groningen [a], Jelle de Koning [a,b], Latifa Lamriti [a], Marcel J. van der Schans [a], Arian C. van Asten [b,c]

[a] TNO Defence, Safety and Security, Dep. CBRN Protection, Lange Kleiweg 137, 2288GJ Rijswijk, the Netherlands
[b] van 't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam, P.O. Box 94157, 1090GD Amsterdam, the Netherlands
[c] CLHC, Amsterdam Center for Forensic Science and Medicine, University of Amsterdam, P.O. Box 94157, 1090GD Amsterdam, the Netherlands

ARTICLE INFO

Keywords:
Chlorine gas
Mass spectrometry
Chemometrics
Likelihood ratio
Forensic chemistry
Chemical warfare agent

ABSTRACT

Chlorine gas (Cl_2) is still being used as a chemical weapon in modern war zones. After an attack with this toxic chemical, verifying its use is challenging due to its reactivity and rapid evaporation. Additionally, only a limited number of selective markers relating to the use of chlorine gas are found. For forensic purposes it is necessary to discriminate Cl_2 from other chlorine containing chemicals. Therefore, the current study aims to use gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) together with machine learning methods to discover selective markers for chlorine gas exposure. Four types of concrete originating from Europe or the Middle East were exposed to various levels of Cl_2 as well as to some commonly used chlorine containing chemicals. After extraction with various solvents, the samples were analyzed. Based on the tentative identification of 32 chlorinated markers with GC–MS and 146 markers with LC-HRMS/MS, principal component analysis (PCA) and linear discriminant analysis (LDA) models were constructed. A clear distinction between concrete samples exposed to bleach and chlorine gas was apparent. For forensic classification, a Bayesian likelihood ratio (LR) model was applied that showed limited rates of misleading evidence and maximum LRs of 0.038 to 93. The markers chloroacetone, dichloroacetone, tetrachlorophenol, and 5-chloro-8-quinolinol were found to be selective for chlorine gas exposure. Their identity was verified by GC–MS/MS and LC-MS/MS in comparison with commercially available reference standards. In conclusion, this study demonstrates the feasibility of chemical profiling in concrete to differentiate among various chlorinating agents.

1. Introduction

Chlorine gas is one of the most studied compounds in chemistry due to its reactive nature and its wide array of applications [1]. However, molecular chlorine in its gaseous state is acutely toxic and can lead to airway blockage, acute lung injury, and in some instances, death [2]. Since its widespread use, chlorine gas can pose a danger to humans when accidentally released into the environment. One of the most fatal industrial accidents occurred in 2022, when a tank filled with 25 tons of chlorine gas fell in the port of Jordan and exploded, leaving 12 people killed and 251 injured [3]. In addition, the intentional deployment of chlorine gas as a chemical warfare agent has caused many victims. The first known malicious use of chlorine gas dates back to World War I, when the German Army deployed 160 tons of chlorine gas in a surprise attack at the frontline over French trenches. This attack killed approximately 1000 French soldiers and injured thousands more [4]. Though the use of chlorine gas as a chemical weapon remains strictly banned by the Chemical Weapons Convention (CWC) its use in war zones continues to persist today. The Organisation for the Prohibition of Chemical Weapons (OPCW) confirmed multiple instances of chlorine gas being used in the Syrian Arab Republic. One such attack in 2018 targeted residential buildings in the city of Douma, resulting in the deaths of 43 civilians and injuring 500 others [5].

Following an attack supposedly involving chlorine gas, samples will be collected at the scene to verify chemical weapon use. This is a challenging task since chlorine gas is reactive and rapidly diffuses. To date, research has largely focused on biomedical samples where chlorinated biomarkers can be found for a longer period of time. Biomarkers such as chlorinated aldehydes, (site-specific) chlorinated tyrosines, chlorinated lipids, trichloromethane, chloroacetone, dichloroacetone, and dichloroacetonitrile were identified as promising markers [6–17]. Also, chlorinated tyrosines, chlorinated dopamines, fenclonine, 5-chlorocytosine,

* Corresponding author.
E-mail address: mirjam.debruin@tno.nl (M. de Bruin-Hoegée).

https://doi.org/10.1016/j.forc.2025.100680
Received 19 December 2024; Received in revised form 15 June 2025; Accepted 16 June 2025
Available online 18 June 2025

and 2-amino-6-chloropurine were identified as persistent chlorine gas markers in vegetation [18,19]. However, in urban areas the availability of plants may be limited. Additionally, in biomedical samples it is difficult to distinguish between endogenous formation of chlorine markers and exogeneous chlorine gas exposure [9]. Another challenge could be the collection of samples of victims due to safety considerations. In such instances, the use of concrete, which is the most abundant building material in the world, could be a promising alternative [20].

Concrete generally consists of 7–15 % cement (main ingredient calcium hydrogen silicate), 14–21 % water, 60–75 % aggregate and often up to 8 % of entrained air [21]. The chemical composition of aggregates varies largely, dependent on the type of minerals, temperature, and mining location. Additionally, admixtures can be added to increase the durability and strength of concrete [22]. Although chloride ions, originating from these admixtures or seawater, have been widely studied in the context of accelerating corrosion of the reinforcing steel structure in concrete [23,24], limited research is available on the effect of chlorine gas on concrete. Recently, the OPCW investigation and identification team described the use of concrete samples as evidence after a suspected chlorine gas attack in Douma, in the Syrian Arab Republic [25]. The detection of highly chlorinated phenols suggested exposure to a strong chlorinating agent, such as chlorine gas. The presence of these markers was confirmed by the research of Hamzah et al. who found different markers for concrete exposed to chlorine gas compared to bleach [26]. However, the most important distinguishing marker tetrachlorophenol (TeCP) was only found in concrete that was older than 70 years or spiked with lignin, a phenolic polymer present in wood. Since the aggregate can be made of a wide range of materials, including waste products, it is unpredictable whether lignin is present in the mixture and consequently form chlorinated phenols after chlorine gas exposure. Therefore, it is crucial to identify alternative markers for chlorine gas exposure that are more universally applicable for forensic investigations. Moreover, there is a need to find markers that can discriminate between chlorine gas exposure and the legitimate use of common chlorine containing chemicals.

Consequently, the aim of the current study is to identify selective markers for chlorine gas exposure in concrete using mass spectrometric techniques together with machine learning methods. Experiments were conducted using four types of concrete originating from various parts of the world. Two types of hardened concrete were used, and two types of cement were investigated. The concrete was exposed to concentrated sodium hypochlorite (NaClO), pool bleach (<15 % NaClO), and household bleach (<5 % NaClO) and three levels of chlorine gas to investigate markers that exclusively relate to chlorine gas exposure and not to chlorine originating from cleaning and disinfection agents. After extraction with various solvents, the samples were analyzed by gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS). Subsequently, the markers were tentatively identified by comparison with reference databases. Afterwards, machine learning methods were applied to reduce the dimensionality of the data and visually identify characteristic markers. The identification of several compounds was verified by commercially available reference standards that were additionally analyzed by gas chromatography tandem mass spectrometry (GC–MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS).

2. Experimental

2.1. Safety

The exposure experiments were conducted in a fume hood and in leak-tight containment by trained analysts. Personal protective equipment, such as safety glasses, lab coats, and gloves, was consistently utilized.

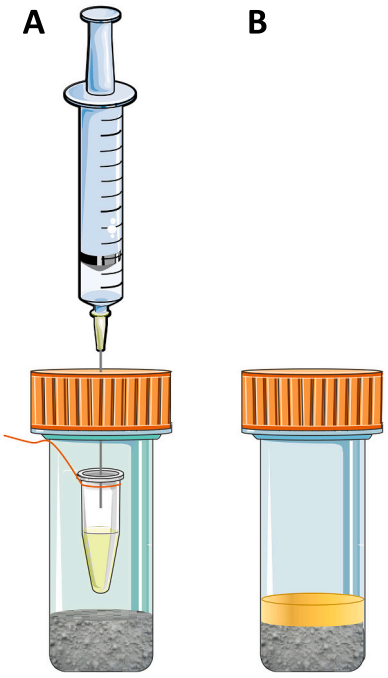

Fig. 1. Schematic experimental set-up for controlled exposure of concrete to A) chlorine gas, and B) bleach.

2.2. Chemicals and materials

Calcium hypochlorite ($Ca(ClO)_2$), ethyl acetate, acetone, formic acid, 5-chloro-8-quinolinol, 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP), dimethylcarbamyl chloride, 5-(hydroxymethyl)uracil, chloroacetyl chloride, 4-chlorophenoxyacetic acid, 2,2,2-trichloroethanol, Dibrom®, fenvalerate, dimethenamid, zoxamide, and 1,3-dichloroacetone were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands), and magnesium sulfate and chloroacetone were acquired from Honeywell Fluka (Loughborough, LE, UK). Acetonitrile (ACN), toluene, hexane, dichloromethane (DCM), and methanol (MeOH) were purchased from Biosolve (Valkenswaard, The Netherlands) and hydrochloric acid (HCl) was obtained from ThermoFisher Scientific (Landsmeer, The Netherlands). The purities of the chemicals exceeded 95 %. Additionally, MilliQ water (SimPak® 1, Merck, Cork, Ireland) was employed. Four types of concrete were obtained from various sources: A) Europe, 2020s, crushed rock, B) Middle East, 2020s, natural gravel, C) Europe, 2023, Sakrete, quick-drying mortar (Praxis, The Netherlands), D) Europe, 2023, Weber quick-drying cement (Hornbach, The Netherlands). Concentrated sodium hypochlorite (10–20 % NaOCl) with 60–185 g/L active chlorine was obtained from Boom (Meppel, The Netherlands). Three commercial household bleaches (< 5 % NaOCl) were obtained from local grocery stores Albert Heijn (AH and Glorix) and Dirk (1deBeste). Pool chlorine containing 12.5–15 % NaOCl was obtained from bol.com (B-care, Smartchim, and Huchem).

2.3. Exposure of concrete

First, two types of hardened concrete were crushed with a mortar into a fine powder. For each sample, 2 g of concrete or cement powder was put in a 20 mL vial. A total of 280 experiments were performed. This included 93 concrete samples exposed to chlorine gas, 103 samples treated with bleach, 66 non-exposed concrete samples (referred to as blanks) and 18 negative samples, where the same method was followed but without the addition of concrete. A more detailed overview of all the

experiments is given in Section S1 of the Supporting Information. The samples were exposed to three concentrations of chlorine gas and seven different types of bleach. A concentration of 50 mg/mL, 500 mg/mL, and 5 g/mL chlorine gas (referred to as chlorine low, mid, and high, respectively), was generated by the reaction of 1, 10, or 100 mg $Ca(ClO)_2$ and 1 mL of 12 M HCl, similar to the method applied by de Bruin-Hoegée et al. [17].

Fig. 1A presents a schematic overview of the setup. It is important to emphasize that this concentration is calculated for a 100 % reaction yield. Most likely a lower concentration was generated since some of the calcium hypochlorite powder was shielded by chlorine gas bubbles and remained unreacted in the vial after the reaction. The concrete was left in the closed vial to react with the generated chlorine gas for two hours. The concrete was also treated with three commercial household bleaches (< 5 % NaOCl), three brands of pool chlorine (12.5–15 % NaOCl), and concentrated NaOCl (10–20 %). A volume of 1 mL bleach was added to 2 g of concrete in a 20 mL vial (Fig. 1B). The concrete was left in the closed vial to react with bleach for two hours.

2.4. Sample preparation

After exposure to either chlorine gas or bleach the samples were prepared for analysis based on the recommended operating procedures for CWC-related analysis [27]. First, the extraction efficiency of various solvents was tested. For GC–MS analysis, ethyl acetate (EA), dichloromethane (DCM), toluene, acetone, methanol (MeOH), and hexane were evaluated. For LC-MS analysis, water, water +1 v% formic acid, water +1 v% formic acid +10 v% MeOH, and DCM was tested. For the latter, the samples were subsequently dried under nitrogen and redissolved in water. A volume of 2 mL solvent was added to the concrete samples and vortexed for 10 s. This was followed by thorough mixing of the samples on a roller mixer (Stuart SRT9, LabMakelaar Benelux) for a period of one hour at 60 rpm. Following mixing, the extraction liquid was transferred to a new vial and subsequently filtered through a 0.45 µm polytetrafluorethylene (PTFE) syringe filter (Alltech, Deerfield, United States). If no clear solution was achieved, the samples were also filtered through a 10 kDa Amicon ultra-centrifugal filter (Merck, Cork, Ireland) at 14,000 rpm for 10 min in an Eppendorf centrifuge (5430R). First, the samples were immediately extracted and processed for further analysis without drying, to avoid the evaporation of valuable markers. However, some water originating from bleach can dissolve in ethyl acetate which can subsequently damage the GC column. To avoid the presence of water in the ethyl acetate extracts for GC–MS analysis, the samples were dehydrated over a column with $MgSO_4$ with a cotton plug. The samples were diluted by a factor of 10 or 100 before analysis with GC–MS, GC–MS/MS, LC-MS/MS, and LC-HRMS/MS as described in section 2.5.

2.5. Chemical analysis

2.5.1. Untargeted screening by GC–MS

The samples were analyzed using an Agilent 7890B gas chromatograph (Ctc Analytics, Zwingen, Switzerland) equipped with an Agilent VF-5 ms column (5 % phenylmethyl polysiloxane, 30 m × 0.25 µm, film thickness 0.25 µm). A volume of 1 µL was injected using a Combi Pal (Ctc Analytics, Zwingen, Switzerland) autosampler. A constant flow of 1 mL/min helium (pressure approximately 48 kPa) was used as carrier gas. The injection was operated in splitless mode at 250 °C. The oven temperature was held for 1 min at 40 °C, then increased at 10 °C/min. to 280 °C and maintained at that temperature for 5 min. Detection was performed with an Agilent 5977A MS operating in electron ionization (EI) mode with a scan range of 25–550 mass units and an ionization potential of 70 eV. The MS transfer line temperature, the source temperature, and the MS quad temperature were set at 275 °C, 230 °C, and 150 °C, respectively. Raw data was loaded into Chemstation (LTS 01.11), and chemicals were subsequently identified with the National Institute of Standards and Technology Mass Spectral Library (NIST11).

2.5.2. Targeted analysis by GC–MS/MS

Analysis was performed on an Agilent 8890 gas chromatograph (Da Vinci, Rotterdam, The Netherlands) with an Agilent VF-5 ms column (5 % phenylmethyl polysiloxane, 30 m × 0.25 mm × 1 µm). The injection volume was 1 µL (PAL RTC autosampler, Da Vinci, Rotterdam, The Netherlands). A constant frow of 1 mL/min helium was used as carrier gas (pressure approximately 51 kPa). The injection was operated in splitless mode at 280 °C. The oven temperature was maintained at 40 °C for 1 min, then ramped at 10 °C/min to 260 °C, and then increased at 10 °C/min to 280 °C, which was maintained for 3 min. The GC system was coupled to an Agilent 7000D triple-quadrupole mass spectrometer, which was operated in EI and multiple reaction monitoring (MRM) mode. The MS transfer line temperature was 275 °C, the source temperature 230 °C, and the MS quad temperatures were set at 150 °C. A flow of 1.5 mL/min nitrogen and a quench flow of 2.25 mL/min helium was used as a collision gas. The solvent delay time was 10 min. The monitored mass transitions were 162 ➔ 98 (15 eV) and 162 ➔ 63 (40 eV) for DCP, 196 ➔ 132 (15 eV) and 196 ➔ 97 (40 eV) for TCP, and 230 ➔ 131 (15 eV) and 230 ➔ 166 (35 eV) for 2,3,5,6-TeCP, 2,3,4,5-TeCP, and 2,3,4,6-TeCP. Raw data was imported into Agilent MassHunter Workstation GC–MS data acquisition (version 10.1.49) and subsequently analyzed in Qualitative Analysis (version 10 build 1.0.01305.0).

2.5.3. Untargeted screening by LC-HRMS/MS

The samples were analyzed with a Thermo Ultimate 3000 ultrahigh-performance liquid chromatography (UHPLC) system (Germering, Germany) equipped with a Waters Acquity HSS T3 C18 column (particle size 1.8 µm, 2.1 × 100 mm). The column temperature was maintained at 30 °C with a flow rate of 100 µL/min (pressure range 400–550 bar). Eluent A consisted of 0.2 v% formic acid in water. Eluent B was composed of 0.2 v% formic acid in acetonitrile. Gradient elution started at 100 % eluent A, ramping to 80 % eluent B in 10 min and holding for 3 min. The system was equilibrated at 100 % eluent A for 7 min. The injection volume was 10 µL. The UHPLC system was coupled to a Thermo Scientific Q Exactive Plus Orbitrap mass spectrometer, which was set to a mass scan range of m/z 50–750 and operated in positive electrospray ionization mode. The capillary voltage was set to 3.5 kV, and the source temperature was maintained at 320 °C, the relative sheath gas nitrogen flow was set at 35. Data was acquired in full scan MS and parallel reaction monitoring (PRM) mode. Based on the results obtained, an inclusion list was established using targeted MS/MS with a collision energy of 35 eV for all compounds.

2.5.4. Targeted analysis by LC-MS/MS

A selection of the representative samples (n = 2, for each type of exposure and concrete) was also analyzed with a Waters (Milford, MA, USA) M-class Acquity ultra-high-pressure liquid chromatographic (UPLC) system equipped with a Waters Acquity HSS T3 C18 column (particle size 1.8 µm, 2.1 × 100 mm,). An extract volume of 5 µL was injected at 8 °C, after which the analysis was performed at room temperature with a gradient flow of 100 µL/min (pressure range 400–550 bar). Eluent A consisted of 0.2 v% formic acid in water. The composition of eluent B was 0.2 v% formic acid in acetonitrile. Gradient elution started at 100 % eluent A, then ramped to 80 % eluent B in 11 min and held for 3 min. Finally, the system was equilibrated at 100 % eluent A for 2 min in preparation for the next analysis. The UPLC system was coupled to a Waters Xevo TQ-S triple-quadrupole mass spectrometer (Milford, MA, USA), equipped with ESI operating in positive ionization mode. A capillary voltage of 3.5 kV was applied. The nitrogen cone gas flow was 150 L/h, and the argon collision gas flow was set to 0.19 mL/min. Data was acquired in selected reaction monitoring (SRM) mode and all compounds were analyzed with a single chromatographic method. The identity of several compounds was verified with a synthetic reference standard by comparing retention times, precursor ion, and characteristic fragment ion m/z values. The monitored mass transitions are given in Section S2 of the Supporting Information.

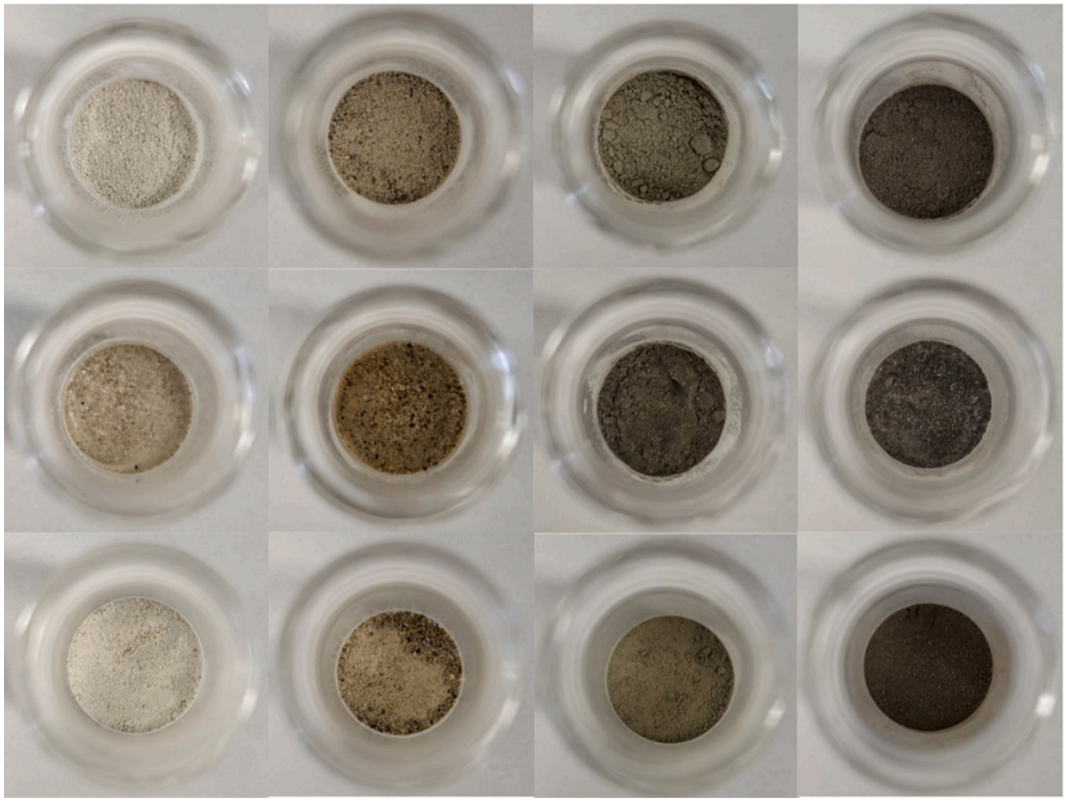

Fig. 2. Blank concrete (top row), concrete immersed in bleach (2nd row) and concrete exposed to a high concentration of chlorine gas (3rd row). From left to right: various types of concrete A, B, C, and D.

2.6. Data analysis

LC-HRMS/MS results were processed with Compound Discoverer 3.3.1.111 (Thermo Scientific). Peak areas were obtained by automatic integration of the extracted ion chromatograms of the identified compounds, after subtraction of a negative control baseline signal of solvent blanks. The masses for the tentative molecular structures were within 5 ppm of the theoretical *m/z*. A substantial fraction of chlorinated chemicals did not match with chemical structures in the Chemspider database and only formulas were presented. However, for many non-chlorinated chemicals a match was found with the database. Subsequently, the non-chlorinated chemicals in the blank were compared to the formulas of unidentified chlorinated chemicals in the treated samples, similar to an earlier published method [19]. In the results, these compounds were marked with [chemical formula] + Cl*. In addition, the chemical should have been identified in at least 26 repetitions (the size of the smallest group) of either the blank, bleach, or chlorine gas samples.

Python 3.9.12 with scikit-learn 1.0.2 and lir 0.1.27 was used for the machine learning analysis, based on an earlier published method [28]. Before applying the models, the data was normalized using the Normalizer function, which rescales the vector for the samples individually to unit norm [29]. For the GC–MS data a PCA model was applied to reduce the dimensionality of the data and visually identify characteristic markers for the various exposure methods. The robustness of PCA was tested by a leave-one-out validation method, which evaluates the effect of removing one sample on the performance of the model. The replicates that were used in this study were all separate experiments and not replicate measurements of the same sample. For the LC-HRMS/MS data an LDA model was constructed to classify the type of exposure. Subsequently, two LDA binary models were constructed from the data to distinguish between unexposed and exposed concrete samples and to separate samples exposed to chlorine gas or bleach. The method was validated by a leave-one-out validation method, similar to the method presented by de Bruin-Hoegée et al. [30]. The LDA plots with Kernel density estimation (KDE) were translated to likelihood ratios (LRs) to express the degree of similarity. To prevent extrapolation errors with a limited sample set, the LR was calibrated by applying an empirical upper and lower bound (ELUB) based on the normalized Bayes error-rate as published by Vergeer et al. [31]. The following hypothesis pair was investigated for the first binary model:

- H_1: The concrete was not exposed to chlorine gas or bleach.
- H_2: The concrete was exposed to either chlorine gas or bleach.

For the second binary model the following hypothesis pair was investigated:

- H_1: The concrete was exposed to chlorine gas.
- H_2: The concrete was exposed to bleach.

3. Results & discussion

3.1. Visual examination of concrete after exposure

Slight changes in the concrete were visible after bleach and chlorine gas exposure (Fig. 2). After immersion with bleach the concrete darkened, but at the same time some white crystals appeared on the surface. A high concentration of chlorine gas did not largely influence the appearance. Only for concrete B, some brown/yellow discoloration was observed on the concrete. Since the effects were minimal, color change is not a clear indicator of chlorine gas versus bleach exposure and cannot be used exclusively as a differentiating feature. This corresponds with the findings of the OPCW Fact-Finding Mission in Syria, where no clear color changes of concrete were reported after the suspected use of chlorine gas as a chemical weapon [25]. However, the visual effects may be useful as indicators of where to sample an incident scene. It should be

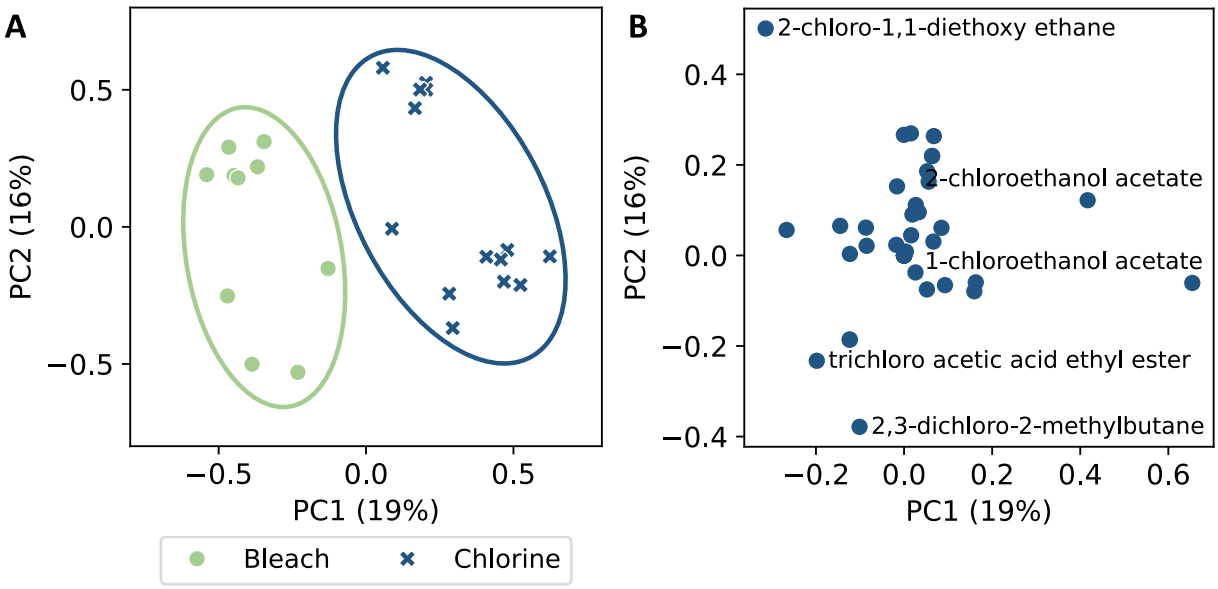

Fig. 3. A) PCA-score plot of different concrete samples extracted with ethyl acetate and analyzed with GC–MS after bleach ($n = 12$) or chlorine gas exposure ($n = 14$). B) PCA-loading plot. The grouping is mainly influenced by PC1, which represents 19 % of the variance and is mainly affected by 1-chloroethanol acetate and 2-chloroethanol acetate. PC2 accounts for 16 % of the variance and is predominantly influenced by 2-chloro-1,1-diethoxy ethane and 2,3-dichloro-2-methylbutane.

noted that crushed concrete was used for the experiments, which is not always present in real-life scenarios when buildings or large pieces of concrete are exposed. Therefore, it is recommended to sample the surface of the concrete or collect the concrete dust that may be formed after an explosion.

3.2. *Classification of chlorinating agent*

GC–MS analysis of concrete samples extracted with organic solvents revealed the highest number of chlorinated markers in the DCM extracts. However, this solvent is less favorable to verify the use of chlorine gas because DCM itself contains chlorine, which can result in false positives. Extraction with ethyl acetate resulted in a higher number of identified markers with GC–MS, compared to toluene, acetone, methanol, and hexane. The latter solvents showed a limited marker variability, where most concrete markers resembled the structure of the extraction solvent. Therefore, ethyl acetate, which resulted in a wide variability of markers, was selected as the solvent of choice for this research. It should be noted that ethyl acetate presents a methodological challenge because of the risk to take up water from the bleach exposed samples, which is destructive for the GC–MS system. This was resolved by dehydrating the samples with $MgSO_4$ before analysis. Fortunately, this sample preparation did not influence the composition of the markers.

In the ethyl acetate extracts a total of 32 chlorinated compounds were tentatively identified (Section S3 of the Supporting Information). This section elaborates on compounds that were not detected in unexposed samples and only visible in the treated samples. Two silane molecules were identified, possibly originating from silane-based additives that are used as water repellent in concrete [32]. Another uncommon marker was dichlorodimethyltin, which may be the reaction product of chlorine gas with cassiterite (SnO_2), a common mineral found in rocks. Both markers were only detected in the chlorine gas exposed concrete samples. There are several markers that have been reported before by Hamzah et al. [26] that are also found in the current study. However, as an exception chloral hydrate, which was identified as a marker for chlorine gas in the earlier study, was only found in bleach samples in the current study. In addition, chloromethylbenzene and dichloromethylbenzene were only detected in bleach exposed samples in the earlier study, while these compounds were only found in concrete samples exposed to chlorine gas and extracted with toluene in the

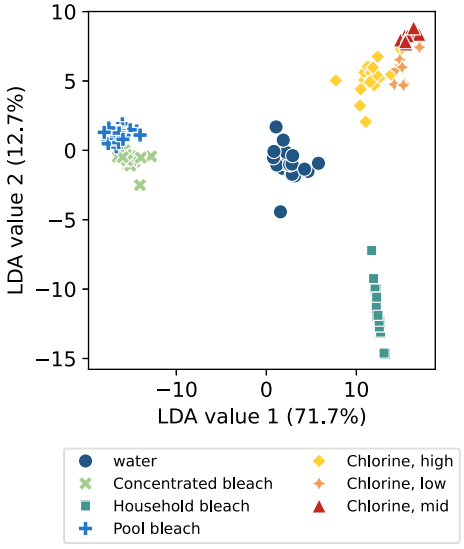

Fig. 4. LDA-score plot for classification of concrete samples exposed to low, mid, and high concentration chlorine gas and household, pool, or concentrated bleach. The first discriminant function (LDA value 1) represents 71.7 % of the total variance and LDA value 2 corresponds to 12.7 % of the total variance.

current study. On the other hand, chlorodimethylbenzene was consistently found as a marker for chlorine gas exposure. The unchlorinated form dimethylbenzene was also found in the blank concrete samples, which confirms the relevance of this compound. It should be noted that no GC-high-resolution-MS analysis was applied in the current study, so the compounds were only identified with library matching and the identification was not verified by the exact mass.

The normalized area of the detected compounds in the ethyl acetate extracts was used to construct a PCA model (Fig. 3). Only compounds were included that were more abundantly present in the exposed samples compared to the blank samples of unexposed concrete. Grouping of the exposure method was evident with full separation of the samples exposed to either chlorine gas or bleach. Fig. 3B shows which compounds mainly contribute to the separation of the exposure methods.

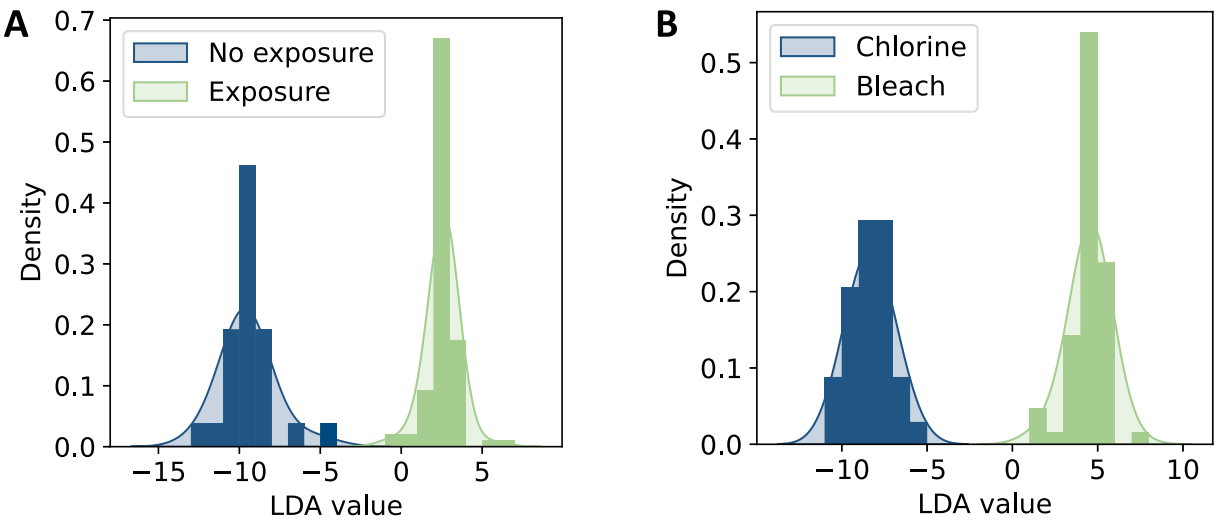

Fig. 5. Distribution of LDA test scores. A) Concrete without exposure or with chlorine gas or bleach exposure. B) Concrete exposed to bleach or chlorine gas. The bars represent the frequency of the measurements adding up to 1 and the distribution is the kernel density estimation with a bandwidth of 1.

The PCA model showed appropriate performance, as demonstrated by leave one out validation (Section S4 of the Supporting Information).

Following data optimization, a list with more than 4500 chlorine-containing chemicals was compiled that were detected by LC-HRMS/MS. The compounds were more abundantly present in the exposed samples compared to the unexposed samples. From this set, 146 compounds were tentatively identified through exact mass measurements, MS/MS spectra, and comparison with spectral libraries. The list of

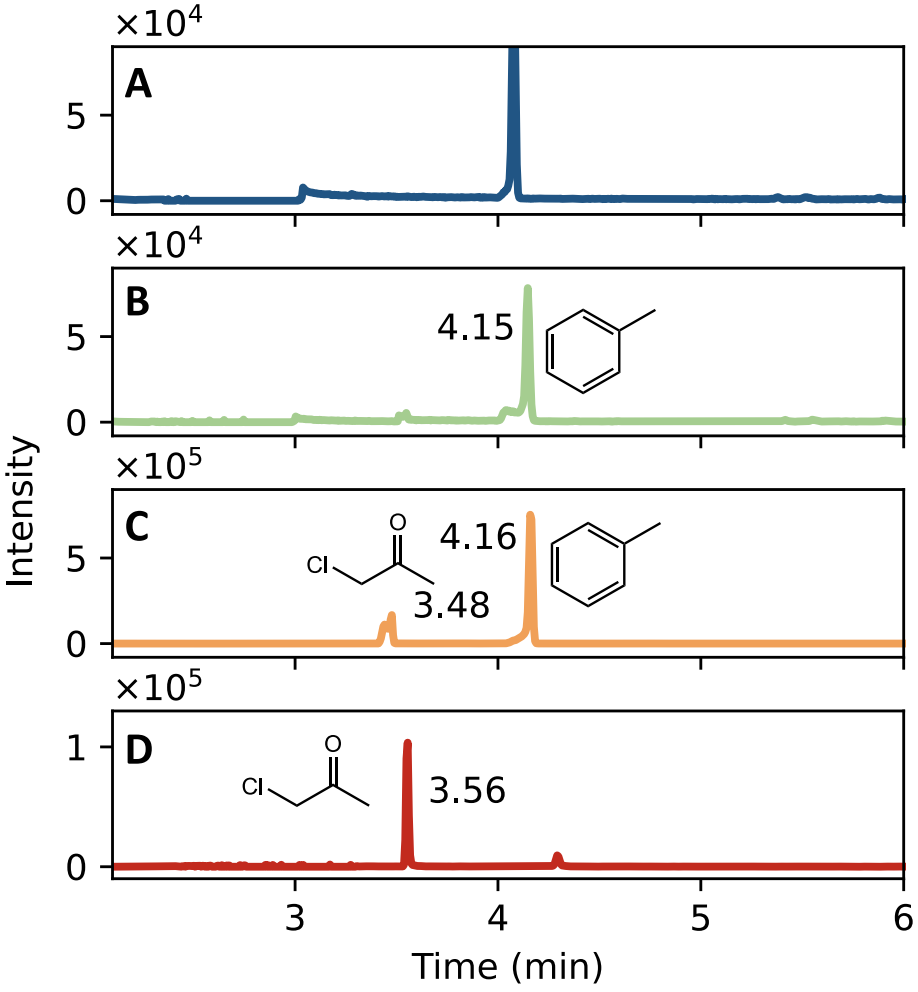

Fig. 6. Extracted ion chromatograms (mass: 92) of chloroacetone at t_r = 3.56 min. A) Sample preparation blank, B) Concrete exposed to concentrated bleach, C) Concrete exposed to a high concentration chlorine gas, D) Commercially available reference standard.

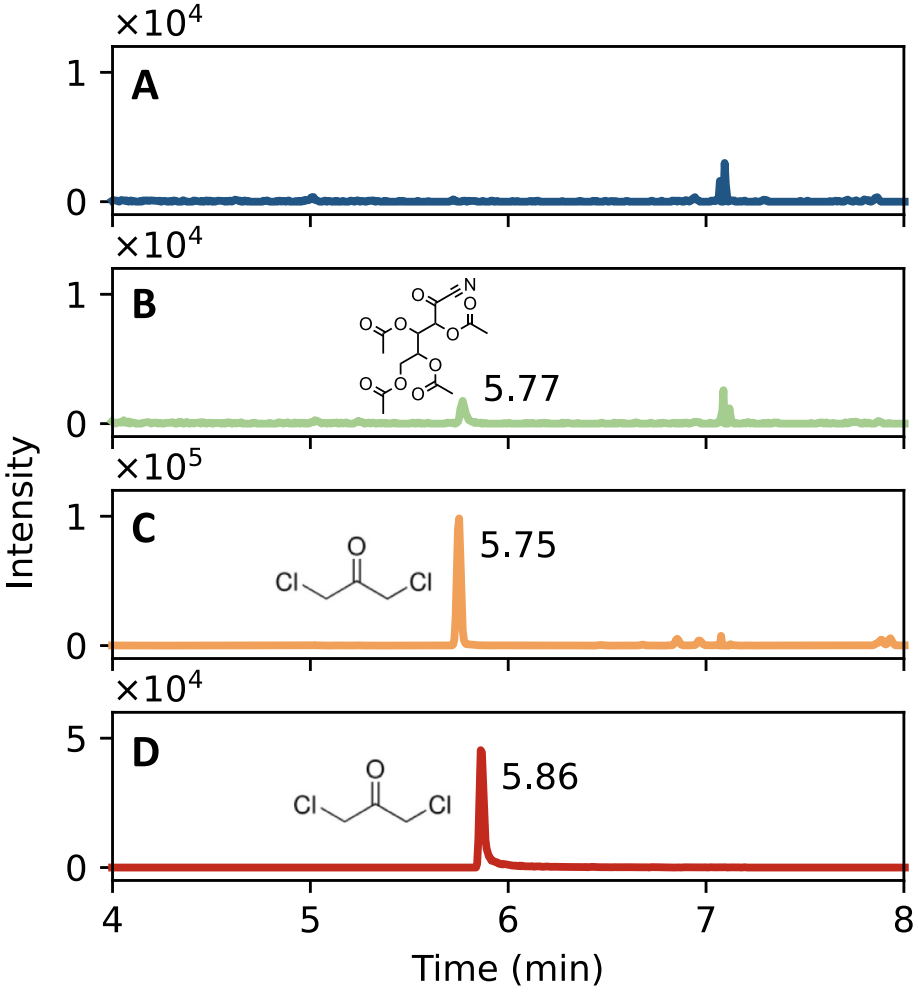

Fig. 7. Extracted ion chromatograms (mass: 126) of 1,3-dichloroacetone at $t_r = 5.86$ min. A) Sample preparation blank, B) Concrete exposed to concentrated bleach, C) Concrete exposed to a high concentration chlorine gas, D) Commercially available reference standard.

chemicals is provided in Section S5 of the Supporting Information. Some chemicals were related to concrete production. For instance, several fatty acids were tentatively identified, which are typical additives of concrete used to increase the fineness of cement [33]. These markers were more abundantly present in the concrete samples treated with bleach. Only a fraction of the compounds (less than 5 %) was detected in either the chlorine gas or bleach exposed samples. This complicates the discrimination process since most markers cannot unambiguously be assigned to the Cl source.

A supervised LDA model was built using the normalized peak area of the compounds identified with LC-HRMS/MS. The score plot of the first two LDA dimensions is presented in Fig. 4. The chlorine gas exposed samples are distinguishable from the bleach exposed samples and water blanks. However, the different types of exposure are not clearly separated. Section S6 of the Supporting Information visualizes major compounds that contribute to the separation of the classes. A disadvantage of this detailed approach with multiple classes is that minor changes in the dataset strongly affect the LDA values. To be able to build a more robust model for classification of the samples, the next section elaborates on a two-step-framework with a binary LDA model (2 classes) for each level, which will be used to calculate likelihood ratios for the considered hypothesis pairs.

Fig. 5A shows a LDA score plot with Kernel density estimations (KDEs). Clear separation was visible of the unexposed materials and the chlorine gas and bleach exposed concrete samples. To validate the model, a leave-one-out validation method was applied. In this way, most samples were correctly classified, except for 2 out of 26 (15 %) false positive outcomes and 7 out of 97 false negative results (7 %). For the samples that were considered to be exposed to a chlorinating agent, the type of exposure was determined next. Fig. 5B presents a LDA score plot with distinct grouping of the samples. LDA values below 2 were clearly assigned to bleach exposed concrete samples and values above to were characteristic for chlorine gas exposed samples. This model was also validated with a leave-one-out validation method. A slightly higher fraction was correctly classified, with only 1 out of 34 chlorine gas exposed samples and 2 out of 63 bleach exposed samples that were erroneously attributed.

Subsequently, a Bayesian approach was applied, to be able to include a measure of similarity and the rarity of the profile. The LDA plots with KDE were translated to likelihood ratios to express the evidential value when considering two opposing hypotheses. Because the plots show almost perfect separation, extremely high likelihood ratio values were obtained. To prevent extrapolation problems due to the limited size of the dataset, the LR range was calibrated by applying ELUB boundaries. In this way LR values of $3.8 * 10^{-2}$ to 93 were obtained for the first model. A high positive LR means that the result is more probable when concrete was exposed to bleach or chlorine gas than when concrete was not exposed to these chlorinating agents. For the second model a range of $1.6 * 10^{-2}$ to 34 was calculated. In this case, an LR value exceeding 1 means that the finding is more probable when concrete was exposed to bleach than when concrete was exposed to chlorine gas. For the calibrated models, no samples were erroneously classified.

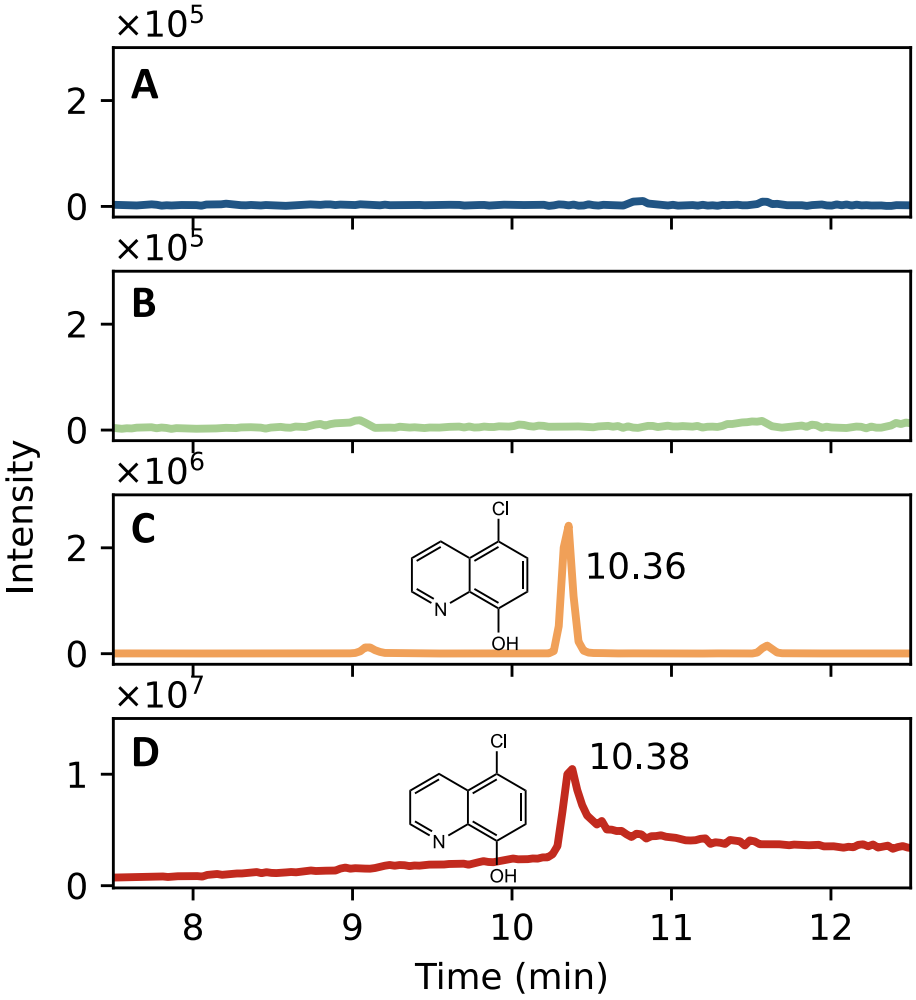

Fig. 8. Extracted ion chromatograms (m/z 180 ➔ 145) of 5-chloro-8-quinolinol at t_r = 10.38 min detected with LC-MS/MS. A) Sample preparation blank, B) Concrete exposed to concentrated bleach, C) Concrete exposed to a high concentration chlorine gas, D) Commercially available reference standard.

3.3. Identification of novel markers

The tentative identification of a selection of markers was verified by comparison with commercially available reference standards. Unfortunately, the tentative identification of dimethylcarbamyl chloride, 5-(hydroxymethyl)uracil, chloroacetyl chloride, 4-chlorophenoxyacetic acid, 2,2,2-trichloroethanol, Dibrom®, fenvalerate, dimethenamid, and zoxamide could not be verified. The retention time and spectra of the reference standard did not match with the automatic identification. This means that the marker was wrongly identified by database comparison and that the marker is most likely another (isomeric) compound that is not in the database. Therefore, this result emphasizes that automatic detection based on database comparisons should be interpreted with caution.

Nevertheless, a total of six tentative identifications could be verified using commercially available reference standards. Five compounds were verified by GC–MS and GC–MS/MS and one marker was confirmed by LC-MS/MS. In the current study, only positive ESI mode was used for LC-MS/MS analysis. To be able to identify more markers, measurements in negative ionization mode can be valuable for analyzing a broader range of compounds [26]. Fig. 6 shows the extracted ion chromatograms of chloroacetone analyzed in the ethyl acetate extract by GC–MS. The marker, which was also visible in the acetone and DCM extract, was only present in chlorine gas exposed concrete samples and absent in non-exposed samples, negative control samples, and concrete exposed to bleach. Since, chloroacetone might form when the solvent acetone reacts with residual Cl_2 in the exposed concrete samples, acetone is not recommended to use as an extraction solvent. Although Fig. 6 shows some variation in retention time, this was within 0.2 min, as recommended in the work instruction for the reporting of the results of the OPCW proficiency tests [34]. The additional peak at a retention time (t_r) of 4.2 min is an isobaric compound with a tentative identification of toluene (NIST library match score: 839). The NIST library match score of chloroacetone in the standard and the chlorine gas exposed samples was above 800. Also, the double chlorinated chemical dichloroacetone was confirmed through the use of a reference standard (Fig. 7). The marker was only visible in concrete exposed to chlorine gas. The library match score of dichloroacetone in the standard and the chlorine gas exposed samples was above 800. The peak eluting at the same time in the bleach exposed samples at t_r 5.8 min was tentatively identified as tetraacetyl-d-xylonic nitrile (NIST library match score: 606). Both chloroacetone and dichloroacetone were consistently present in the two tested concrete types, A and B, but only in samples exposed to high concentrations of chlorine gas.

In addition, the markers DCP, TCP, and TeCP, that were also previously documented [26], were confirmed by GC–MS/MS analyses of commercial reference standards. Linear calibration curves are shown in Section S7 of the Supporting Information, with a limit of detection of 1 ng/mL for DCP and TCP and 50 ng/mL for TeCP. Both DCP and TCP were found in the toluene, DCM, methanol, and ethyl acetate extracts of concrete exposed to chlorine gas and bleach. Interestingly, TeCP was only found in the DCM extracts of chlorine gas exposed samples.

One marker was identified by LC-MS/MS in comparison with commercially available reference standards. Fig. 8 shows the extracted ion chromatograms of 5-chloro-8-quinolinol (m/z 180 ➔ 145). The marker was present in all types of concrete exposed to the various concentrations of chlorine gas ($n = 2$ for each category) and absent in non-exposed samples, negative control samples, and concrete exposed to bleach. A compound with an exact mass corresponding to the unchlorinated form 8-quinolinol, was detected in the untreated concrete samples. However, no database match was found, so comparison with reference standards is needed to confirm its identify.

4. Conclusions

In the present study novel markers of exposure to chlorine gas were identified in concrete, using GC–MS, LC-HRMS/MS, and application of machine learning methods. PCA of GC–MS data highlighted tentatively identified compounds, such as chloral hydrate, silane-based additives, and possible reaction products of cassiterite. The presence of chlorodimethylbenzene was found to be characteristic for chlorine gas exposed samples, in accordance with earlier research. Remarkably, this compound was not found in the bleach exposed samples and the unchlorinated form dimethylbenzene was identified in untreated concrete. After the chemometric analysis, a more straightforward method was developed by comparison with commercially available reference standards. The markers chloroacetone, dichloroacetone, tetrachlorophenol were distinctive for chlorine gas exposed concrete samples and were not present in unexposed and bleach exposed samples. However, tetrachlorophenol was only detected in samples extracted with DCM. In future research, it can be beneficial to process more sample material, to increase the marker concentrations and enable identification in other extracts as well. Additionally, linear discriminant analysis of LC-HRMS/MS data revealed several characteristic compounds including fatty acids which are typical additives in concrete production. The identification of 5-chloro-8-quinolinol was unambiguously verified by LC-MS/MS analysis of the corresponding reference standard. This marker was solely detected in concrete exposed to chlorine gas and could be a valuable marker to verify chlorine gas exposure. Since the preliminary identification of several other markers proved to be indecisive, further studies could expand this research by verifying more tentatively identified markers with reference standards. Future research can also investigate real-life scenarios, where large concrete blocks instead of crusted concrete samples are exposed to chlorinating agents. Shorter exposure times could also be explored to more accurately reflect real-world conditions. In conclusion, selective markers were identified that can be used to provide valuable intelligence information regarding the applied chlorinating agent. Ultimately, well-validated likelihood ratio models could facilitate forensic classifications for war crime investigations in the International Criminal Court.

CRediT authorship contribution statement

Mirjam de Bruin-Hoegée: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Bence Dallos:** Validation, Software, Methodology, Investigation, Formal analysis. **Tomas van Groningen:** Methodology, Investigation. **Jelle de Koning:** Methodology, Investigation. **Latifa Lamriti:** Methodology, Investigation. **Marcel J. van der Schans:** Writing – review & editing, Supervision, Conceptualization. **Arian C. van Asten:** 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.

Acknowledgements

This work was partially funded by the Dutch Ministry of Foreign Affairs. This work is part of the Forensic Attribution for CWA INtelligence (FACING) project, a collaboration between the Van 't Hoff Institute for Molecular Sciences (HIMS) of the University of Amsterdam and TNO Defence, Safety & Security. The FACING project is financed by the DO-AIO fund of the Dutch Ministry of Defence.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2025.100680.

Data availability

Data will be made available on request.

References

[1] B.J. Finlayson-Pitts, Chlorine chronicles, Nat. Chem. 5 (2013) 724, https://doi.org/10.1038/nchem.1717.
[2] C.W. White, J.G. Martin, Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models, Proc. Am. Thorac. Soc. 7 (2010) 257–263, https://doi.org/10.1513/pats.201001-008SM.
[3] Reuters, Chlorine gas leak kills 12, injures 251 at Jordan port. reuters.com/world/middle-east/four-dead-70-injured-toxic-gas-leak-jordans-aqaba-port-state-tv-2022-06-27, 2022. (Accessed 12 June 2025).
[4] G.J. Fitzgerald, Chemical warfare and medical response during world war I, Am. J. Public Health 98 (2008) 611–625, https://doi.org/10.2105/ajph.2007.11930.
[5] OPCW, Director-General's Statement on the Third Report by the OPCW Investigation and Identification Team, (2023). https://www.opcw.org/sites/default/files/documents/2023/02/Director-General's Statement on the Third Report by the OPCW Investigation and Identification Team.pdf (accessed July 11, 2023).
[6] B.G. Pantazides, B.S. Crow, J. Quiñones-González, J.W. Perez, J.A. Harvilchuck, J. J. Wallery, T.C. Hu, J.D. Thomas, R.C. Johnson, T.A. Blake, Development of a clinical assay to measure chlorinated tyrosine in hair and tissue samples using a mouse chlorine inhalation exposure model, Anal. Bioanal. Chem. 413 (2021) 1765–1776, https://doi.org/10.1007/s00216-020-03146-x.
[7] S.V. Martz, M. Wittwer, C.W. Tan-Lin, C.G. Bochet, M. Brackmann, C. Curty, Influence of chlorinating agents on the formation of stable biomarkers in hair for the retrospective verification of exposure, Anal. Chem. 94 (2022) 16579–16586, https://doi.org/10.1021/acs.analchem.2c01867.
[8] P. Hemström, A. Larsson, L. Elfsmark, C. Åstot, L -α-Phosphatidylglycerol chlorohydrins as potential biomarkers for chlorine gas exposure, Anal. Chem. 88 (2016) 9972–9979, https://doi.org/10.1021/acs.analchem.6b01896.
[9] M. de Bruin-Hoegée, I.M. van Damme, T. van Groningen, D. van Der Riet-Van Oeveren, D. Noort, A.C. van Asten, Elucidation of in vitro chlorinated tyrosine adducts in blood plasma as selective biomarkers of chlorine exposure, Chem. Res. Toxicol. 35 (2022) 1070–1079, https://doi.org/10.1021/acs.chemrestox.2c00053.
[10] T. Nishio, Y. Toukairin, T. Hoshi, T. Arai, M. Nogami, Development of an LC–MS/MS method for quantification of 3-chloro-L-tyrosine as a candidate marker of chlorine poisoning, Legal Med. 53 (2021) 101939, https://doi.org/10.1016/j.legalmed.2021.101939.
[11] S. Jonasson, R. Magnusson, H. Wingfors, Å. Gustafsson, G. Rankin, L. Elfsmark, L. Mörén, Potential exhaled breath biomarkers identified in chlorine-exposed mice, J. Anal. Toxicol. 48 (2024) 171–179, https://doi.org/10.1093/jat/bkae007.
[12] P. Hemström, B. Jugg, R. Watkins, S. Jonasson, L. Elfsmark, S. Rutter, C. Åstot, P. Lindén, Phospholipid chlorohydrins as chlorine exposure biomarkers in a large animal model, Toxicol. Lett. 391 (2024) 32–38, https://doi.org/10.1016/j.toxlet.2023.11.008.
[13] P. Lindén, S. Jonasson, P. Hemström, L. Ålander, A. Larsson, L. Ågren, L. Elfsmark, C. Åstot, Nasal lavage fluid as a biomedical sample for verification of chlorine exposure, J. Anal. Toxicol. 46 (2022) 559–566, https://doi.org/10.1093/jat/bkab069.
[14] T. Nishio, Y. Toukairin, T. Hoshi, T. Arai, M. Nogami, Determination of 3-chloro-L-tyrosine as a novel indicator of chlorine poisoning utilizing gas chromatography-mass spectrometric analysis, Legal Med. 47 (2020) 101782, https://doi.org/10.1016/j.legalmed.2020.101782.
[15] B.S. Crow, J. Quiñones-González, B.G. Pantazides, J.W. Perez, W.R. Winkeljohn, J. W. Garton, J.D. Thomas, T.A. Blake, R.C. Johnson, Simultaneous measurement of 3-chlorotyrosine and 3,5-dichlorotyrosine in whole blood, serum and plasma by isotope dilution HPLC-MS-MS, J. Anal. Toxicol. 40 (2016) 264–271, https://doi.org/10.1093/jat/bkw011.
[16] S. Achanta, M.A. Gentile, C.J. Albert, K.A. Schulte, B.G. Pantazides, B.S. Crow, J. Quiñones-González, J.W. Perez, D.A. Ford, R.P. Patel, T.A. Blake, M.D. Gunn, S. E. Jordt, Recapitulation of human pathophysiology and identification of forensic biomarkers in a translational model of chlorine inhalation injury, Am. J. Phys. Lung Cell. Mol. Phys. 326 (2024) L482–L495, https://doi.org/10.1152/ajplung.00162.2023.

[17] S.L. Boles, B.G. Pantazides, J.W. Perez, M.R. Sternberg, B.S. Crow, T.A. Blake, Establishing population values for chlorine exposure in the United States (2015–2016) using 2 chlorine biomarkers, 3-chlorotyrosine and 3,5-dichlorotyrosine, J. Appl. Lab. Med. 9 (2024) 342–349, https://doi.org/10.1093/jalm/jfad114.
[18] M. de Bruin-Hoegée, L. Lamriti, J.P. Langenberg, R.C.M. Olivier, L.F. Chau, M. J. van der Schans, D. Noort, A.C. van Asten, Verification of exposure to chemical warfare agents through analysis of persistent biomarkers in plants, Anal. Methods 15 (2023) 142–153, https://doi.org/10.1039/d2ay01650h.
[19] M. de Bruin-Hoegée, M.J. van der Schans, J.P. Langenberg, A.C. van Asten, Biomarker profiling in plants to distinguish between exposure to chlorine gas and bleach using LC-HRMS/MS and chemometrics, Forensic Sci. Int. 358 (2024) 112022, https://doi.org/10.1016/j.forsciint.2024.112022.
[20] Global Cement and Concrete Association (GCCA), Concrete - the world's most widely used material - targets carbon neutral future. https://gccassociation.org/news/concrete-the-worlds-most-widely-used-material-targets-carbon-neutral-future/, 2020. (Accessed 20 July 2023).
[21] The Portland Cement Association, Applications of Cement. https://www.cement.org/cement-concrete/applications-of-cement/, 2024. (Accessed 4 September 2024).
[22] A.M. Neville, Chapter 5. Admixtures, in: Pearson Eduction (Ed.), Prop. Concr., 5th ed., 2011, pp. 821–904.
[23] F. Pruckner, O.E. Gjørv, Effect of CaCl2 and NaCl additions on concrete corrosivity, Cem. Concr. Res. 34 (2004) 1209–1217, https://doi.org/10.1016/j.cemconres.2003.12.015.
[24] D. Sun, Z. Cao, C. Huang, K. Wu, G. De Schutter, L. Zhang, Degradation of concrete in marine environment under coupled chloride and sulfate attack: a numerical and experimental study, Case Stud. Constr. Mater. 17 (2022) e01218, https://doi.org/10.1016/j.cscm.2022.e01218.
[25] OPCW, Third Report by the OPCW Investigation and Identificaiton Team Pursuant to Paragraph 10 of Decision C-SS-4/DEC.3 "Addressing the Threat from Chemical Weapons Use" Douma (Syrian Arab Republic) – 7 APRIL 2018. https://www.opcw.org/sites/default/files/documents/2023/01/s-2125-2023%28e%29.pdf, 2023. (Accessed 28 April 2023).
[26] N. Hamzah, K. Höjer Holmgren, C. Åstot, M.J. van der Schans, L. de Reuver, P. Vanninen, Chlorinated organic compounds in concrete as specific markers for chlorine gas exposure, J. Hazard. Mater. 459 (2023), https://doi.org/10.1016/j.jhazmat.2023.132332.
[27] P. Vanninen, Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament, University of Helsinki, 2023.
[28] M. de Bruin-Hoegée, G.J. de Bruin, Python Code for Biomarker Profiling in Plants, 2023, https://doi.org/10.5281/zenodo.10227405.
[29] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, E. Duchesnay, Scikit-learn: machine learning in Python, J. Mach. Learn. Res. 12 (2011) 2825–2830, https://doi.org/10.48550/arXiv.1201.0490.
[30] M. de Bruin-Hoegée, R. Corzo, P.D. Zoon, P. Vergeer, J. Schoorl, M.J. van der Schans, D. Noort, A.C. van Asten, Evaluating the strength of evidence of elemental profiling of polymers with LA-ICP-MS, Forensic Chem. 38 (2024) 100570, https://doi.org/10.1016/j.forc.2024.100570.
[31] P. Vergeer, A. van Es, A. de Jongh, I. Alberink, R. Stoel, Numerical likelihood ratios outputted by LR systems are often based on extrapolation: when to stop extrapolating? Sci. Justice 56 (2016) 482–491, https://doi.org/10.1016/j.scijus.2016.06.003.
[32] H. Herb, A. Gerdes, G. Brenner-Weiß, Characterization of silane-based hydrophobic admixtures in concrete using TOF-MS, Cem. Concr. Res. 70 (2015) 77–82, https://doi.org/10.1016/j.cemconres.2015.01.008.
[33] A. Tugrul Albayrak, M. Yasar, M.A. Gurkaynak, I. Gurgey, Investigation of the effects of fatty acids on the compressive strength of the concrete and the grindability of the cement, Cem. Concr. Res. 35 (2005) 400–404, https://doi.org/10.1016/j.cemconres.2004.07.031.
[34] OPCW, Technical Secretariat, Work Instruction for the Reporting of the results of the OPCW Biomedical Proficiency Tests, 2021.