Chemosphere 262 (2021) 128288

Contents lists available at ScienceDirect

Chemosphere

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

Transformation of short-chain chlorinated paraffins by the bacterial haloalkane dehalogenase LinB – Formation of mono- and di-hydroxylated metabolites

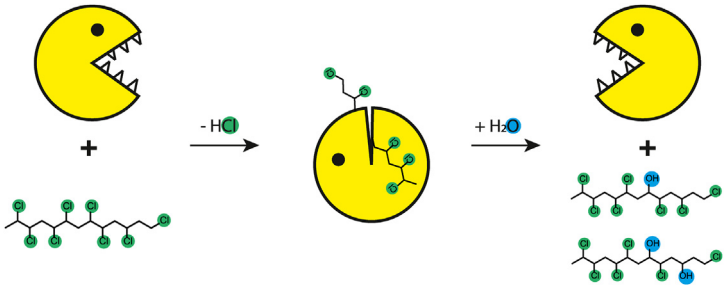
Marco C. Knobloch [a, b, *], Lena Schinkel [c], Iris Schilling [c], Hans-Peter E. Kohler [c], Peter Lienemann [d], Davide Bleiner [a, b], Norbert V. Heeb [a]

[a] Laboratory for Advanced Analytical Technologies, Swiss Federal Institute for Materials Science and Technology Empa, Überlandstrasse 129, 8600, Dübendorf, Switzerland
[b] Department of Chemistry, University of Zürich, Winterthurerstrasse 190, 8057, Zürich, Switzerland
[c] Swiss Federal Institute of Aquatic Research and Technology Eawag, Überlandstrasse 129, 8600, Dübendorf, Switzerland
[d] Zürich University of Applied Sciences ZHAW, Einsiedlerstrasse 3, 8820, Wädenswil, Switzerland

HIGHLIGHTS

- Chlorinated paraffins are transformed by the enzyme LinB.
- Mono- and di-hydroxylated transformation products could be identified.
- Mathematical deconvolution was used to obtain non-interfered data.
- Mono- and di-hydroxylated products were separated from starting material by LC.
- Preferential conversion of lower chlorinated CPs was induced by LinB.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 May 2020
Received in revised form
4 September 2020
Accepted 5 September 2020
Available online 11 September 2020

Handling Editor: J. de Boer

Keywords:
Haloalkane dehalogenase LinB
Chlorinated olefins (COs)
Chlorinated paraffins (CPs)
Hydroxylated chlorinated paraffins
Persistent organic pollutants (POPs)

ABSTRACT

Short-chain chlorinated paraffins (SCCPs) are listed as persistent organic pollutants (POPs) under the Stockholm Convention. Such substances are toxic, bioaccumulating, transported over long distances and degrade slowly in the environment. Certain bacterial strains of the *Sphingomonadacea* family are able to degrade POPs, such as hexachlorocyclohexanes (HCHs) and hexabromocyclododecanes (HBCDs). The haloalkane dehalogenase LinB, expressed in certain *Sphingomonadacea*, is able to catalyze the transformation of haloalkanes to hydroxylated compounds. Therefore, LinB is a promising candidate for conversion of SCCPs. Hence, a mixture of chlorinated tridecanes was exposed *in vitro* to LinB, which was obtained through heterologous expression in *Escherichia coli*. Liquid chromatography mass spectrometry (LC-MS) was used to analyze chlorinated tridecanes and their transformation products. A chloride-enhanced soft ionization method, which favors the formation of chloride adducts $[M+Cl]^-$ without fragmentation, was applied. Mathematical deconvolution was used to distinguish interfering mass spectra of paraffinic, mono-olefinic and di-olefinic compounds. Several mono- and di-hydroxylated products including paraffinic, mono-olefinic and di-olefinic compounds were found after LinB exposure. Mono- (rt = 5.9–6.9 min) and di-hydroxylated (rt = 3.2–4.5 min) compounds were separated from starting material (rt = 7.7–8.5 min) by reversed phase LC. Chlorination degrees of chlorinated tridecanes

* Corresponding author. Laboratory for Advanced Analytical Technologies, Swiss Federal Institute for Materials Science and Technology Empa, Überlandstrasse 129, 8600, Dübendorf, Switzerland.
E-mail address: marco.knobloch@empa.ch (M.C. Knobloch).

https://doi.org/10.1016/j.chemosphere.2020.128288

increased during LinB-exposure from $n_{Cl} = 8.80$ to 9.07, indicating a preferential transformation of lower chlorinated ($Cl_{<9}$) tridecanes. Thus, LinB indeed catalyzed a dehalohydroxylation of chlorinated tridecanes, tridecenes and tridecadienes. The observed hydroxylated compounds are relevant CP transformation products whose environmental and toxicological effects should be further investigated.

1. Introduction

1.1. Short-chain chlorinated paraffins (SCCPs) are persistent organic pollutants (POPs)

Chlorinated paraffins (CPs) are high production volume chemicals (>1 million t/y), widely used as plasticizers and flame retardants in plastic materials and as metal working fluids (Glüge et al., 2016; van Mourik et al., 2016). CPs are classified according to their carbon chain-length into short-chain (SCCPs, $C_{10\text{-}13}$), medium-chain (MCCPs, $C_{14\text{-}17}$), long-chain (LCCPs, $C_{18\text{-}21}$) and very long-chain CPs (vLCCPs, $C_{22\text{-}30}$) (UNEP, 2016). They are produced as technical mixtures with variable chain length and chlorine proportion m_{Cl} of 30–70% (w/w) (Glüge et al., 2016). Technical CPs are mixtures of thousands of constitutional isomers with different carbon chain lengths and chlorination degrees (Tomy, 2009; Fiedler, 2010). SCCPs are now considered as persistent, bioaccumulating and toxic, showing considerable long-range transport potential (Iozza et al., 2009a; Vorkamp and Rigét, 2014; Zeng et al., 2015; Li et al., 2016; Liu et al., 2016; Yuan et al., 2019). In 2017, SCCPs have been listed in the Stockholm Convention as persistent organic pollutants (POPs) and their use is restricted in many countries (UNEP, 2017). MCCPs fulfill the criteria for a restriction in the European Union and are currently under evaluation (Glüge et al., 2018; ECHA, 2019). The environmental and toxicological impact of LCCPs is widely unknown (Schinkel et al., 2018a; de Wit et al., 2020).

1.2. Biotransformation of persistent organic pollutants

Because POPs are persistent and many POPs are ubiquitous in the environment, biota is steadily exposed to these substances, albeit respective concentrations might be low. In 2007, hexachlorocyclohexanes (HCHs), including γ-HCH, which was marketed under the brand name Lindane, were classified as POPs (UNEP, 2009). While γ-HCH was the active insecticide, all other HCH stereoisomers were found to be inactive (Willett et al., 1998). Consequently, estimated 1.9 million tons of HCH-containing waste material have been deposed on dump sites in various countries (Vijgen et al., 2011).

From several HCH-contaminated soils of such dump sites, bacteria of the *Sphingobium* family could be isolated, which adopted the ability to transform HCHs (Pal et al., 2005). These bacteria strains express various enzymes, which can transform HCHs (Senoo and Wada, 1989; Nagata et al., 1999; Lal et al., 2010), but also other POPs, such as hexabromocyclododecanes (HBCDs) (Heeb et al., 2017).

Potential biotransformation reactions of POPs include halide substitution (S_N2-like) and elimination (E2-like) reactions. In S_N2-like reactions, an atom or functional group is substituted by a nucleophilic atom or functional group (Müller and Lingens, 1986; Vogel et al., 1987). Whereas in E2-like reactions two substituents are removed from a molecule (Vogel et al., 1987; Hirschorn et al., 2007; Lal et al., 2010).

For example, the dehydrogenase LinA2 expressed in certain *Sphingomonadace* catalyzes the elimination of hydrogen halides, transforming HCHs and HBCDs to respective unsaturated and dehalogenated metabolites (Lal et al., 2006; Heeb et al., 2014). Recently, it was shown that LinA2 also catalyzes HCl-elimination reactions of CPs, forming chlorinated olefins (COs) (Heeb et al., 2019).

The haloalkane dehalogenase LinB is another enzyme expressed by several *Sphingomonadace*. Haloalkane dehalogenases are also expressed by other bacteria and catalyze substitution reactions of halide substituents with hydroxyl groups (Copley, 1999). As shown in Fig. 1, LinB catalyzes dehalohydroxylation reactions of HCHs and HBCDs to respective mono- and di-hydroxylated products (Raina et al., 2007; Heeb et al., 2013). The crystal structure of LinB from *Sphingomonas paucimobilis* UT26 has been obtained and the catalytic site has been identified (Oakley et al., 2002; Streltsov et al., 2003). Aspartate (Asp108), glutamate (Glu132) and histidine (His272) form a catalytic triade, in which aspartate acts as the nucleophile, releasing a halide (Prokop et al., 2003; Heeb et al., 2013; Manna and Dybala-Defratyka, 2014).

HCHs, HBCDs and CPs are all polyhalogented compounds containing several sp^3-hybridized secondary halide groups (-CHX-). In addition, CPs may also contain primary halide groups ($-CH_2X$). Due to these structural similarities, it was hypothesized that the haloalkane dehalogenase LinB may also convert CPs to hydroxylated products as shown in Fig. 1C. Recently, it was demonstrated that rice cells can metabolize CPs and form transformation products including olefinic and hydroxylated compounds (Chen et al., 2020).

1.3. Challenging analysis of CPs and their transformation products

Because of their high production volumes and broad usage, it is likely that CPs are released to the environment and are transformed by abiotic and biotic processes. Thus, it is important to study the environmental fate of CPs and their transformation. CPs are commonly analyzed by chromatographic methods coupled to a mass spectrometer (van Mourik et al., 2018). The available one-dimensional liquid- and gas-chromatographic methods are insufficient to separate CP homologues (van Mourik et al., 2018). This results in coelution of CP isomers in broad chromatographic peaks (Korytár et al., 2005; Iozza et al., 2009b; Perkons et al., 2019). Furthermore, isotope clusters of different CP homologues can interfere severely, even at high mass resolution of 10′000 (Schinkel et al., 2017). To minimize such interferences, single-chain CP mixtures can be used to study transformation processes (Schinkel et al., 2018a). The presence of chlorinated mono-olefins (COs) and di-olefins (CdiOs), which are the expected dehydrochlorination products of CPs, in available CP materials further complicates the analysis. Mass spectra of COs and CdiOs strongly overlap with those of CPs (Schinkel et al., 2018b; Heeb et al., 2019). Mathematical deconvolution procedures, relying on linear combinations of theoretical isotope clusters, were developed to resolve mass interference of different CPs (Bogdal et al., 2015), and of CPs, COs and CdiOs (Schinkel et al., 2018c). With these methods both, abiotic and biotic transformation reactions of CPs to COs can be observed (Schinkel et al., 2017, Schinkel et al., 2018a; Heeb et al., 2019).

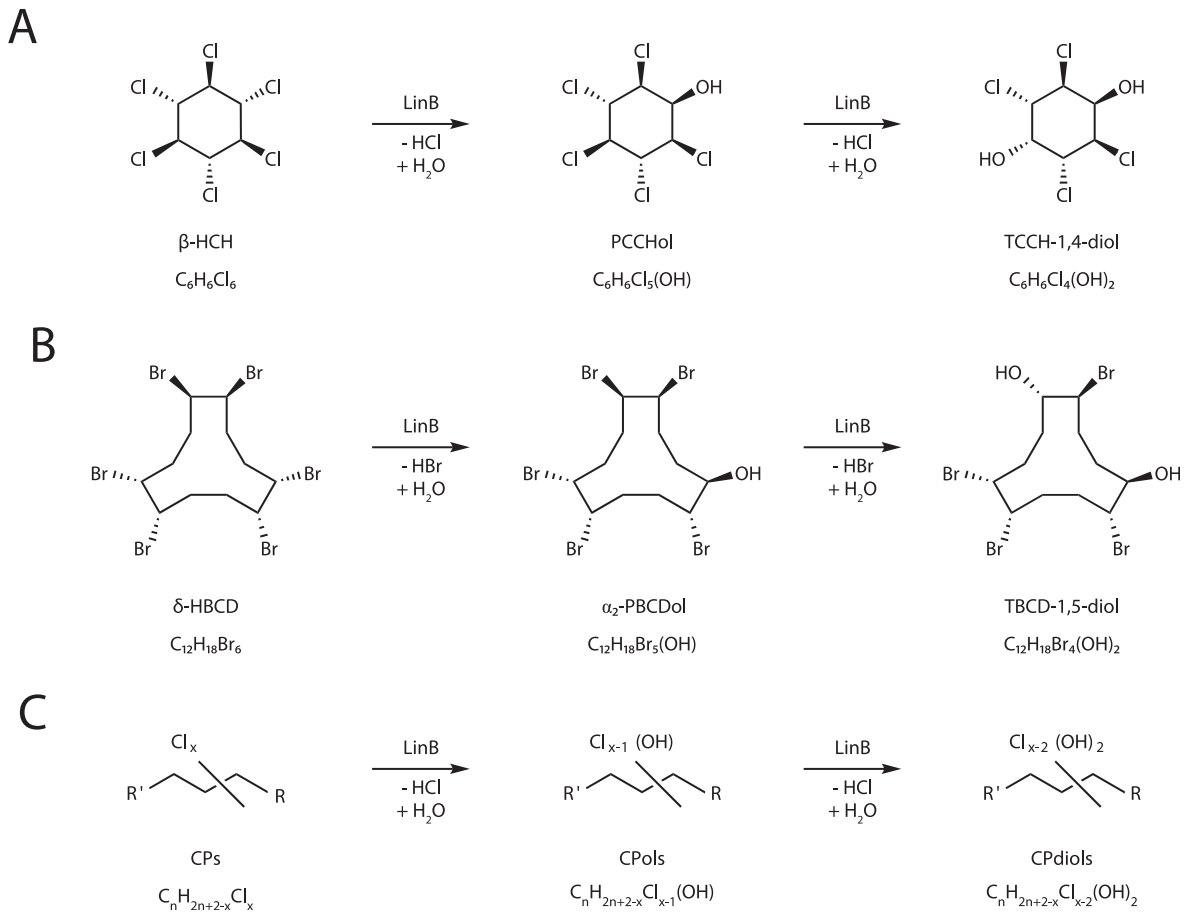


Fig. 1. LinB-catalyzed dehalohydroxylation of β-HCH (A), δ-HBCD (B) and CPs (C). The S_N2-like dehalohydroxylation of β-HCH (A) leads to mono- and dihydroxylated products (Raina et al., 2007). A pentachlorocyclohexanol (PCCHol) and a tetrachlorocyclohexanediol (TCCH-1,4-diol) are formed in consecutive reactions. The stepwise transformation of δ-HBCD leads to a respective pentabromocyclododecanol (α_2-PBCDol) and a tetrabromocyclododecanediol (TBCD-1,5-diol) as shown (B) (Heeb et al., 2013). Accordingly, we hypothesized that CPs may be transformed by LinB-catalyzed dehalohydroxylation reactions to form mono- (CPols) and di- (CPdiols) hydroxylated products (C).

1.4. Study aims and applied analytical methods

Here, we report on the enzymatic transformation of chlorinated tridecanes with the bacterial enzyme LinB of *Sphingobium indicum* to mono- and di-hydroxylated products. A chromatographic separation of non-hydroxylated and hydroxylated material was achieved. Mathematical deconvolution procedures were used to resolve interfering mass spectra of paraffinic, mono-olefinic and di-olefinic material of non-, mono- and dihydroxylated compounds. With it, we could answer the question if LinB does catalyze S_N2-like substitution- or E2-like elimination reactions or both. A mass spectrometric and chromatographic characterization of various hydroxylated CP transformation products was established. These findings show the importance of hydrolytic transformation reaction of CPs in bacteria.

2. Experimental

2.1. Substrates, chemicals and buffer materials

A mixture of chlorotridecanes with a chlorination degree m_{Cl} of 65.18% (Dr. Ehrenstorfer, Augsburg, Germany) was used as substrate. δ-HBCD (Empa, Dübendorf, Switzerland) was used as internal standard (IS). Methanol, ethyl acetate, dichloromethane (all from Biosolve, Valkenswaard, Netherlands), acetone (Merck, Darmstadt, Germany) and purified water (Milli-Q Reference A+, 18.2 MΩcm) were used. Glycine, tris(hydroxymethyl)amino methane (tris), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride and imidazole (all from Sigma-Aldrich, Buchs, Switzerland) were used as buffer materials. L-(+)-arabinose (Sigma-Aldrich) was used to induce enzyme expression.

2.2. Enzyme cloning, protein expression and purification

Codon-optimized pDEST17 vector (John Oakeshott, CSIRO, Canberra, Australia) with the histidine-tagged LinB-gene from *Sphingobium indicum* B90A was cloned into *Escherichia coli* BL21-AI bacteria. These gene-modified bacteria were grown at 37 °C and 120 rounds per minute (rpm) for 16 h in lysogeny broth (LB, 8 mL) which contained ampicillin (150 μg/L). The strain was incubated in LB (800 mL) to reach an optical density (OD_{600}) of 0.5. To induce LinB expression, L-(+)-arabinose was added (2 g/L). After further incubation at 30 °C for 3 h, an OD_{600} of 1.9 was reached. Cells were harvested by centrifugation (Herolab UniCen, Germany) at 10 000 rpm (4 °C) and stored at −20 °C.

Cells were taken up in buffer A (NaH_2PO_4 44 mM, NaCl 300 mM, imidazole 10 mM) and cell lysis was induced by ultrasonification (Sonoplus HD 3200, Bandelin, Berlin, Germany). The suspension was loaded onto an affinity column (BabyBio Ni-NTA, BioWorks, Uppsala, Sweden) and purified by liquid chromatography (ÄKTA FPLC, GE Life Science, England). Buffers A and B (NaH_2PO_4 44 mM, NaCl 300 mM, imidazole 300 mM) were used as eluents. The flow

rate was 5 mL/min. For the first 19 min the proportion of buffer A was 100%, which was then decreased within 5 min to 0% and kept for additional 8 min. Fractions were collected and analyzed by sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) and Coomassie blue staining. Fractions containing >95% pure LinB (visual inspection) were combined and concentrated by ultrafiltration through a 10 kDa filter (Amicon, Ceniprep, USA) at 4000 g and 4 °C for 7 min. A solution of purified LinB enzyme with a concentration of 1.75 mg/mL was obtained. The protein concentration was measured with a photometrical method (Nanodrop ND-1000, Thermo Scientific).

2.3. Enzymatic incubation, workup and controls

A chlorotridecane mixture in cyclohexane (10 ng/μL) was transferred in brown glass vials and concentrated to dryness by an N_2 stream. The residue was taken up with acetone, mixed with 2x buffer (glycine 364 mM, tris 50 mM, pH 8.3), diluted with water and finally LinB solution was added. Total CP concentration at start was 2 μg/mL, concentrations of acetone (10 vol%), LinB enzyme (100 μg/mL), tris (25 mM) and glycine (182 mM) are indicated. Buffer control samples (500 μL) with CPs but without enzyme were taken at start (t = 0 h) and after 144 h exposure at room temperature at 100 rpm. Immediately after collecting samples, 500 μL ethyl acetate

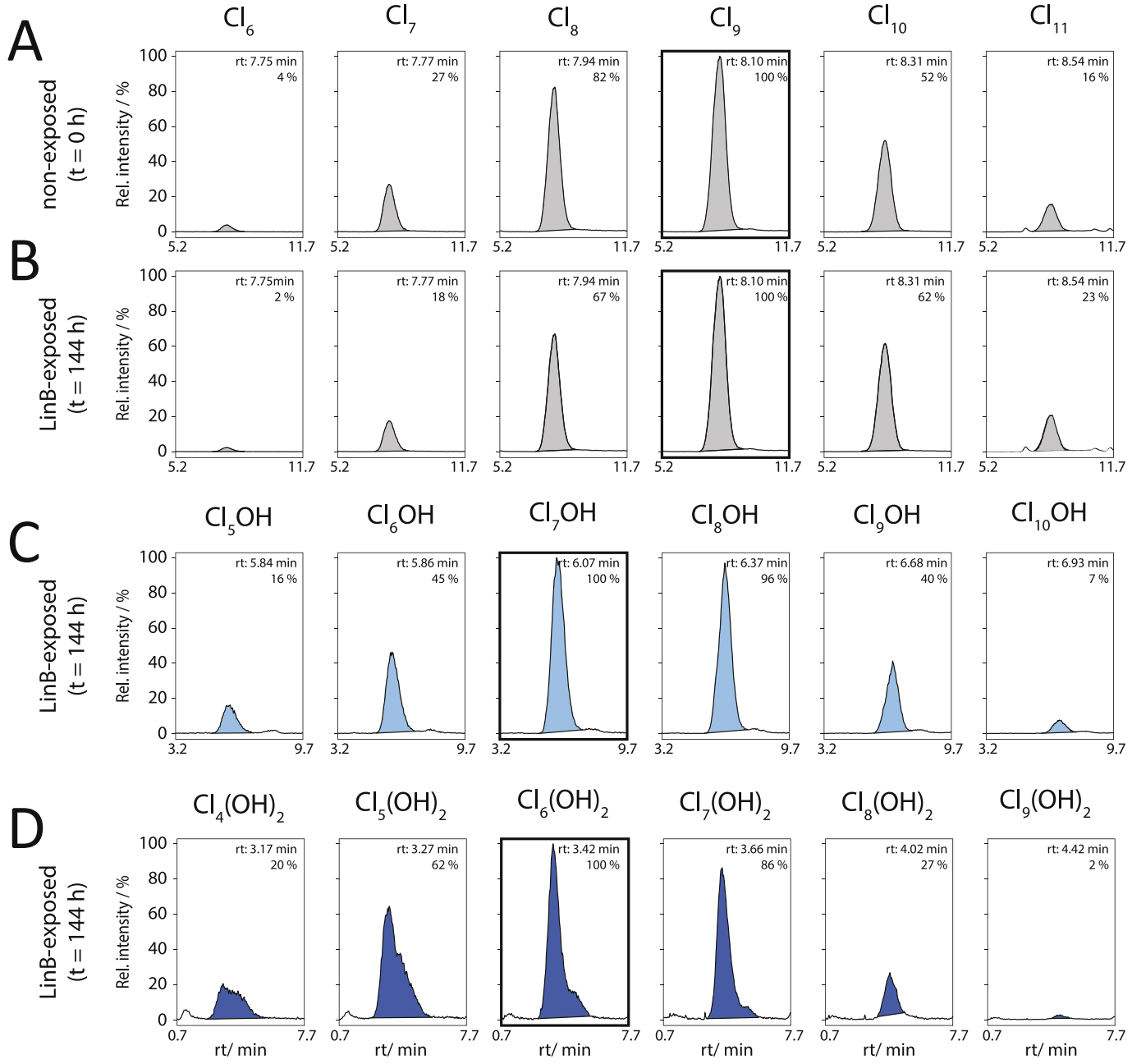


Fig. 2. Extracted ion chromatograms (EICs) of non-exposed and LinB-exposed samples. The UHPLC-APCI-QTOF-MS method, which supports chloride adduct $[M+Cl]^-$ formation, was applied and selected ions of respective isotope cluster were monitored. Chromatographic peaks of hexa- to undeca-chlorotridecanes before (A, gray) and after (B, gray) LinB-exposure are shown. EICs of the LinB-exposed sample show the formed products penta- to deca-chlorotridecanols (C, light blue) and tetra- to nona-chlorotridecanediols (D, dark blue). Peaks are scaled to the most abundant congener (bold frame) in each class of compounds and respective percentages are depicted. The average retention time (rt) is given. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

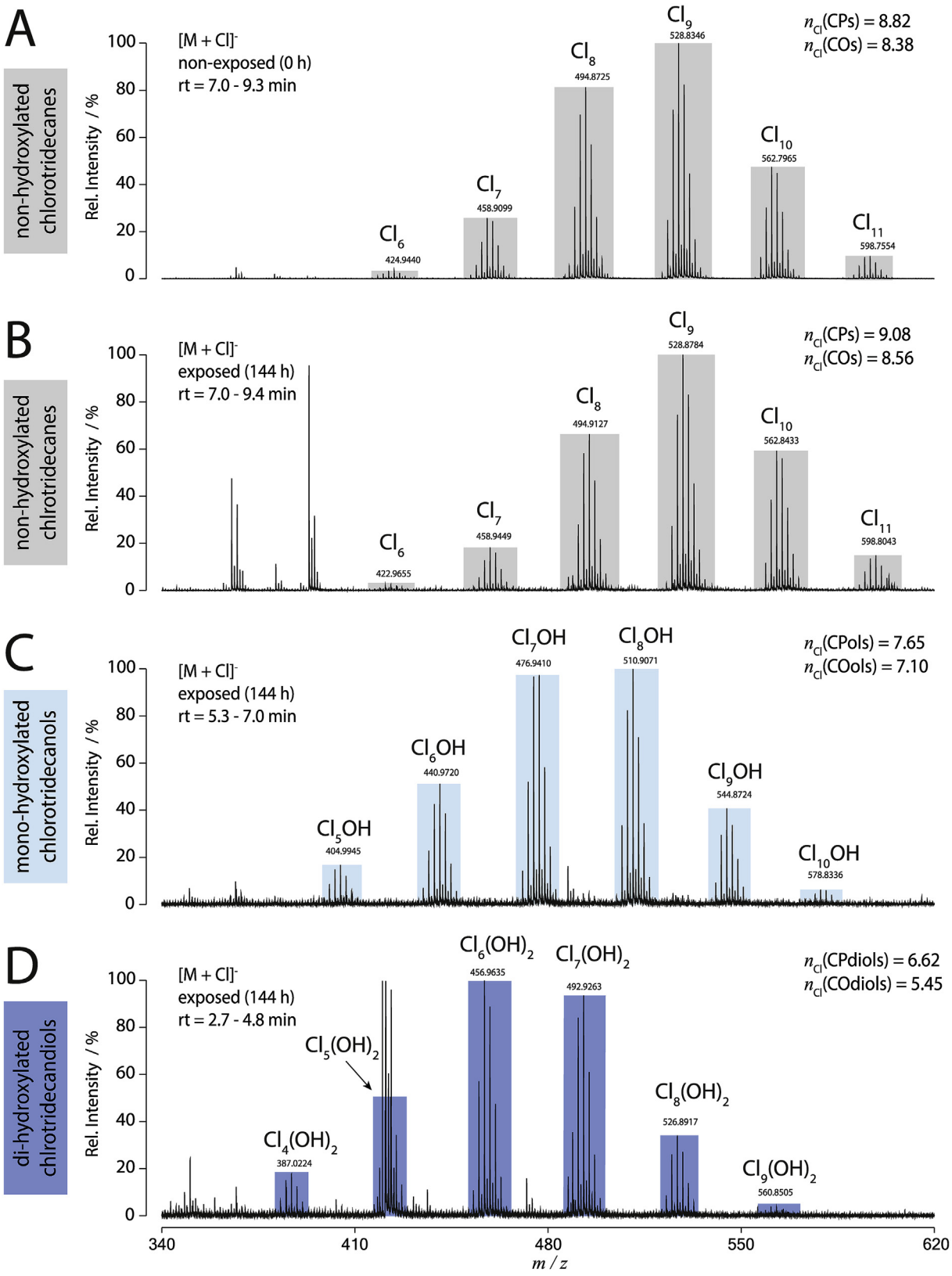


Fig. 3. LC-APCI-QTOF mass spectra (R≈ 8000) of non-exposed and LinB-exposed material. The chlorine-enhanced APCI method leads to the formation of chloride-adduct ions $[M+Cl]^-$. Mass spectra were acquired by integrating chromatographic peaks in respective retention time ranges. Non-hydroxylated material eluted from a C_{18}-reverse phase column during 7.0–9.3 min. Spectra of non-exposed (A) and LinB-exposed (B) materials are compared. Hexa- (Cl_6) to undeca- (Cl_{11}) chlorinated homologues are highlighted in gray. Mono-hydroxylated transformation products (C, light blue) eluted earlier, during 5.3–7.0 min. Penta- (Cl_5) to deca- (Cl_{10}) chlorinated mono-hydroxylated transformation products were detected. Even di-hydroxylated transformation products (D, dark blue) are present in the LinB-exposed sample. Tetra- (Cl_4) to nona- (Cl_9) chlorinated di-hydroxylated homologues eluted earliest, during 2.7–4.8 min. Calculated average chorine numbers of paraffinic n_{Cl}(CP) and mono-olefinic n_{Cl}(CO) materials and their hydroxylated transformation products are also given. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was added to stop transformation reactions (Heeb et al., 2013). Samples were homogenized and stored at −20 °C. Internal standard (δ-HBCD, 50 ng) was added. The organic supernatant was removed after liquid-liquid extraction with ethyl acetate (3 × 500 μL), combined and concentrated to dryness with an N_2 stream. Residues were dissolved in methanol (100 μL) and analyzed twice by LC-MS. Control and blank samples were treated analogously.

2.4. Chemical analysis using LC-APCI-QTOF-MS

Starting material and hydroxylated transformation products were separated by liquid chromatography (LC, Agilent 1290 Infinity) using a C_{18} reverse-phase column (Agilent, SB-C18 RRHD, 50 mm × 3 mm, 1.8 μm, 50 °C). Water (eluent A) and a mixture of methanol/dichloromethane (eluent B, 9/1) were used as eluents with a gradient (60% B for 1 min, 60−98% B in 7 min, 98% B for 2 min, 98-60% B in 1 min). An atmospheric pressure chemical ionization (APCI) source was used. Infusion of dichloromethane supports the formation of chloride adduct ions $[M+Cl]^-$ in the APCI module (Zencak et al., 2003). A quadrupole time-of-flight mass spectrometer (QTOF-MS, Agilent 6520, Santa Clara, CA, U.S.A.) in the negative ion mode was used to monitor CPs and related compounds. The recorded MS data was extracted with Mass Hunter B.07.00 (Agilent Technologies, USA). A previously published deconvolution method was applied to resolve interferences of paraffinic, mono-olefinic and di-olefinic compounds (Bogdal et al., 2015; Yuan et al., 2016; Schinkel et al., 2018c). Simulated mass spectra were obtained with enviPat (Loos et al., 2015). A detailed description of the deconvolution method is provided as supporting information (Fig. S1).

3. Results and discussion

3.1. Chromatographic characterization of CP transformation products

Fig. 2 shows extracted ion chromatograms (EICs) of non-exposed (A) and LinB-exposed (B) starting material and respective mono- (C) and di- (D) hydroxylated transformation products. Average retention times and extracted *m/z* values are given in Table S1 (SI). Hexa-, hepta-, octa-, nona-, deca- and undeca-chlorinated homologues eluted on average in 7.75, 7.77, 7.94, 8.10, 8.31 and 8.54 min under the used LC-conditions (C_{18}-RP). Lower chlorinated homologues elute earlier than higher chlorinated ones. Nona-chlorinated material is most prominent in both, non-exposed (Fig. 2A) and exposed (Fig. 2B) samples. After LinB-exposure, relative peak intensities of hepta- and octa-chlorinated homologues are slightly lower compared to those before exposure.

Fig. S11 shows EICs of non-exposed staring material, control (buffer-exposed, 144 h) and procedure blank samples. CPs and CP transformation products could not be detected in the blank sample. Exposure to buffer only did not affect the CP homologue distribution and the signal intensities. We therefore conclude that observed effects in LinB-exposed samples are due to enzyme-catalyzed transformations.

Fig. 2C demonstrates that penta-, hexa-, hepta-, octa-, nona- and deca-chlorinated alcohols eluted in just 5.84, 5.86, 6.07, 6.37, 6.68, and 6.93 min, on average. This is 1.5−2 min earlier than homologues of the respective starting material. Relative peak intensities show that hepta- and octa-chlorinated alcohols are the most prominent transformation products.

Fig. 2D displays EICs of tetra-, penta-, hexa-, hepta-, octa- and nona-chlorinated diols with average retention times of 3.17, 3.27, 3.42, 3.66, 4.02 and 4.42 min. Thus, dihydroxylated transformation products elute 4.0−4.5 min before the starting material and 2.5−3.0 min before mono-hydroxylated compounds.

The chromatographic study shows that CP transformation products elute in more polar conditions than their precursors. Thus, substituting chlorine atoms with hydroxyl groups results in more polar compounds. The proposed transformation mechanism of CPs with LinB would lead to such hydroxylated products (Fig. 1C).

3.2. Mass spectrometric characterization of CPs and their hydroxylated transformation products

Fig. 3 shows mass spectra of non-exposed and LinB-exposed (144 h) hexa-to undeca-chlorotridecanes (A, B). The applied soft ionization method (APCI) supports the formation of chloride adduct ions $[M+Cl]^-$. Mass spectra show that nona-chlorotridecanes (Cl_9) are most abundant in both, non- and exposed samples. In the starting material, Cl_8-homologues are more prominent than Cl_{10}-homologues. Accordingly, Cl_7-homologues are more prominent than Cl_{11}-ones. After LinB-exposure, Cl_{10}-and Cl_{11}-homologues are nearly as prominent as Cl_8- and Cl_7−homologues, respectively. Thus, exposure to LinB results in a slight shift to a higher chlorination degree in the remaining CP material, indicating a preferred degradation of lower chlorinated material by the LinB enzyme.

It is assumed that also mono- and di-hydroxylated CPs form chloride adducts $[M+Cl]^-$ under the applied MS conditions. Mass spectra of the exposed sample include mono- (Fig. 3C) and di- (Fig. 3D) hydroxylated compounds. These metabolites are expected for the proposed enzymatic dehalohydroxylation mechanism of CPs (Fig. 1C). Penta- up to deca-chlorinated tridecanols were found as first-generation products, with highest abundances of hepta- (35%) and octa- (35%) chlorotridecanols. Furthermore, tetra- up to nona-chlorinated tridecanediols were found as second-generation products, with highest abundances of hexa- (32%) and hepta- (40%) chlorotridecanediols.

3.3. Mass interferences of CPs and CP transformation products

3.3.1. The paraffin-, mono-olefin- and di-olefin-problem

Fig. 4 (left) shows calculated mass spectra of octachlorinated di-olefinic (A), mono-olefinic (B) and paraffinic (C) compounds as chloride adducts $[M+Cl]^-$ at a mass resolution of 10′000. An overlay (Fig. 4D) and close-ups (Fig. 4E) of these mass spectra indicate severe interferences of paraffinic, mono-olefinic and di-olefinic compounds. These compounds cannot be separated by liquid chromatography. For example, to distinguish chloride-adduct ions of CPs with a formula of $[^{12}C_{13}H_{20}^{35}Cl_7^{37}Cl_2]^-$ at $m/z = 494.8697$ (Fig. 4C) from those of COs with a formula of $[^{12}C_{13}H_{20}^{35}Cl_6^{37}Cl_3]^-$ at $m/z = 494.8511$ (Fig. 4B) requires a mass resolution of R > 26′600. In our case, with an instrument operated at R ≈ 8′000, a mathematical deconvolution procedure was used to calculate paraffinic, mono-olefinic and di-olefinic proportions in interfered mass spectra for individual homologues. Fig. S1 (SI) gives an overview of the applied deconvolution procedure.

3.3.2. Mass interferences with hydroxylated transformation products

The right panels of Fig. 4 show calculated mass spectra of heptachloro-tridecenediols (F, COdiols) and heptachloro-tridecanediols (G, CPdiols), which both interfere with octachloro-tridecanes (H, CPs). An overlay (I) and close-ups (J) of these simulated mass spectra reveal interferences of spectra of higher chlorinated paraffins with those of paraffinic and mono-olefinic diols. A mass spectrometric resolution of R > 67′500 is necessary to resolve interferences of CPs with the formula $[^{12}C_{13}H_{20}^{35}Cl_8^{37}Cl_1]^-$ at *m/*

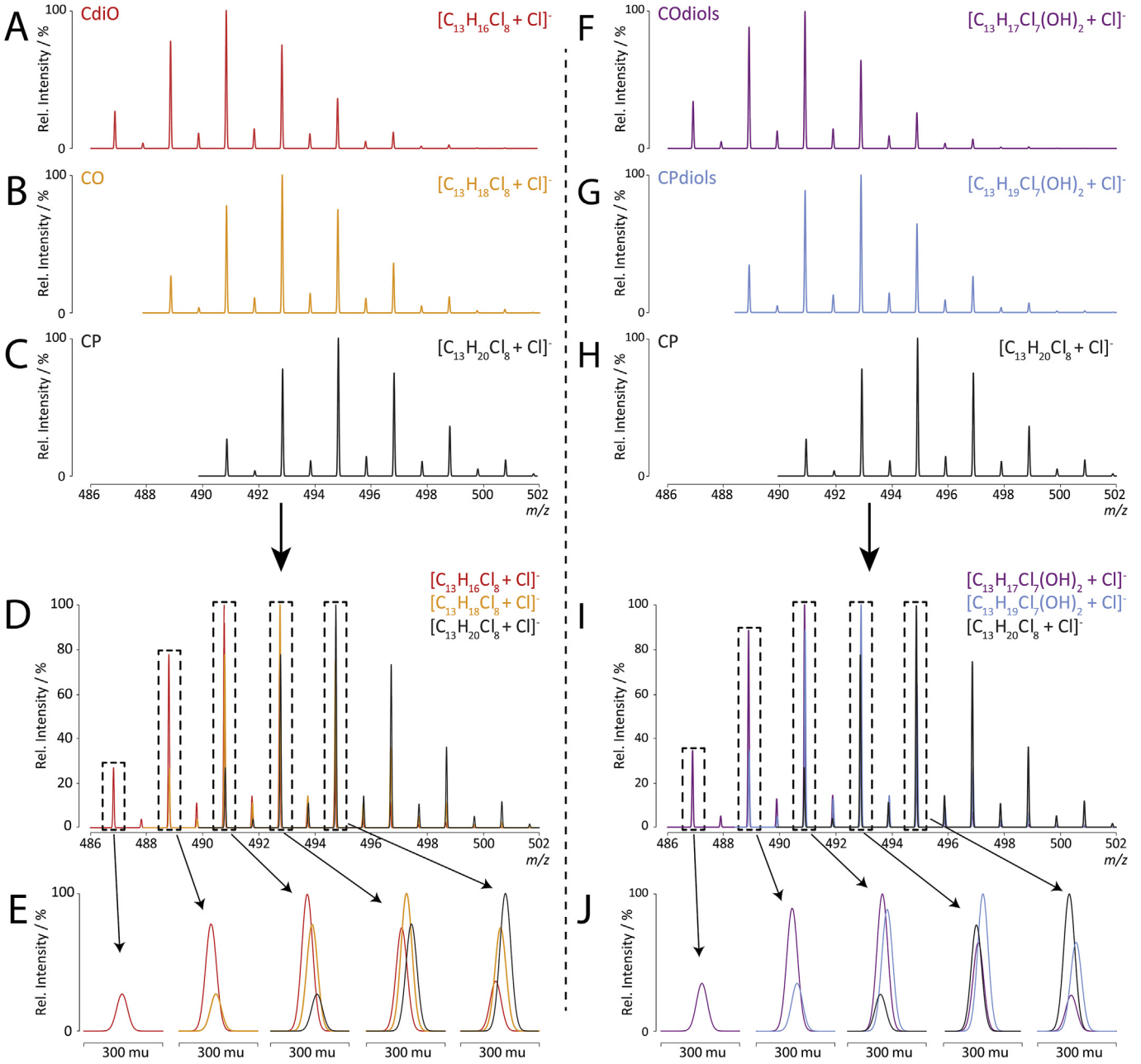


Fig. 4. Simulated mass spectra of interfered CPs and CP-transformation products. Isotope clusters correspond to respective chloride adduct ions $[M+Cl]^-$. Isolated mass spectra (R = 10′000) of interfering compounds are given in A-C and F–H. Spectra of octachlorotridecadienes (CdiOs, A), octachlorotridecenes (COs, B) and octachlorotridecanes (CPs, C) strongly overlap, as do heptachlorotridecenediols (COdiols, F), heptachlorotridecanediols (CPdiols, G) and octachlorotridecanes (CPs, H). An overlayed spectrum (D) of interfering isotope cluster of CPs, COs and CdiOs shows strong interferences. Zooms (E) reveal non-evenly distributed interferences. Paraffinic compounds (H) also interfere with dihydroxylated compounds (F, G). An overlayed (I) spectrum of chlorinated paraffins with dihydroxylated saturated and unsaturated material (F, G) shows these interferences. Respective zooms (J) also reveal variable contributions of interfering COdiols (F), CPdiols (G) and CPs (H).

$z = 492.8727$ (Fig. 4H) and COdiols of $[^{12}C_{13}H_{18}^{35}Cl_7^{37}Cl_2(OH)_2]^-$ at $m/z = 492.8800$ (Fig. 4F). The postulated enzymatic substitution of one chlorine atom with a hydroxyl-group (Fig. 1C) results in a mass difference of ≈ 18 u. Therefore, the mass spectra of the mono-hydroxylated transformation products CPols, COols and CdiOols are not expected to interfere with those of non-hydroxylated CPs, COs and CdiOs. However, substitutions of two chlorine atoms with two hydroxyl-groups result in a mass difference of ≈ 36 u. This mass shift leads to severe interferences of the mass spectra of di-hydroxylated transformation products with those of non-hydroxylated compounds as shown in Fig. 4. However, the presented LC method can separate di-, mono- and non-hydroxylated compounds. Nevertheless, spectra acquired with no or insufficient chromatographic and mass spectrometric resolution can be affected by these interferences if samples contain both, non- and dihydroxylated compounds.

Besides these interferences, one has to expect that also mass spectra of hydroxylated CPs interfere with those of mono-olefinic and di-olefinic compounds. Applying the mathematical deconvolution procedure, the paraffinic and olefinic fraction can also be obtained for alcohols and diols. However, there is considerable ambiguity about the chemical nature of such oxygenated

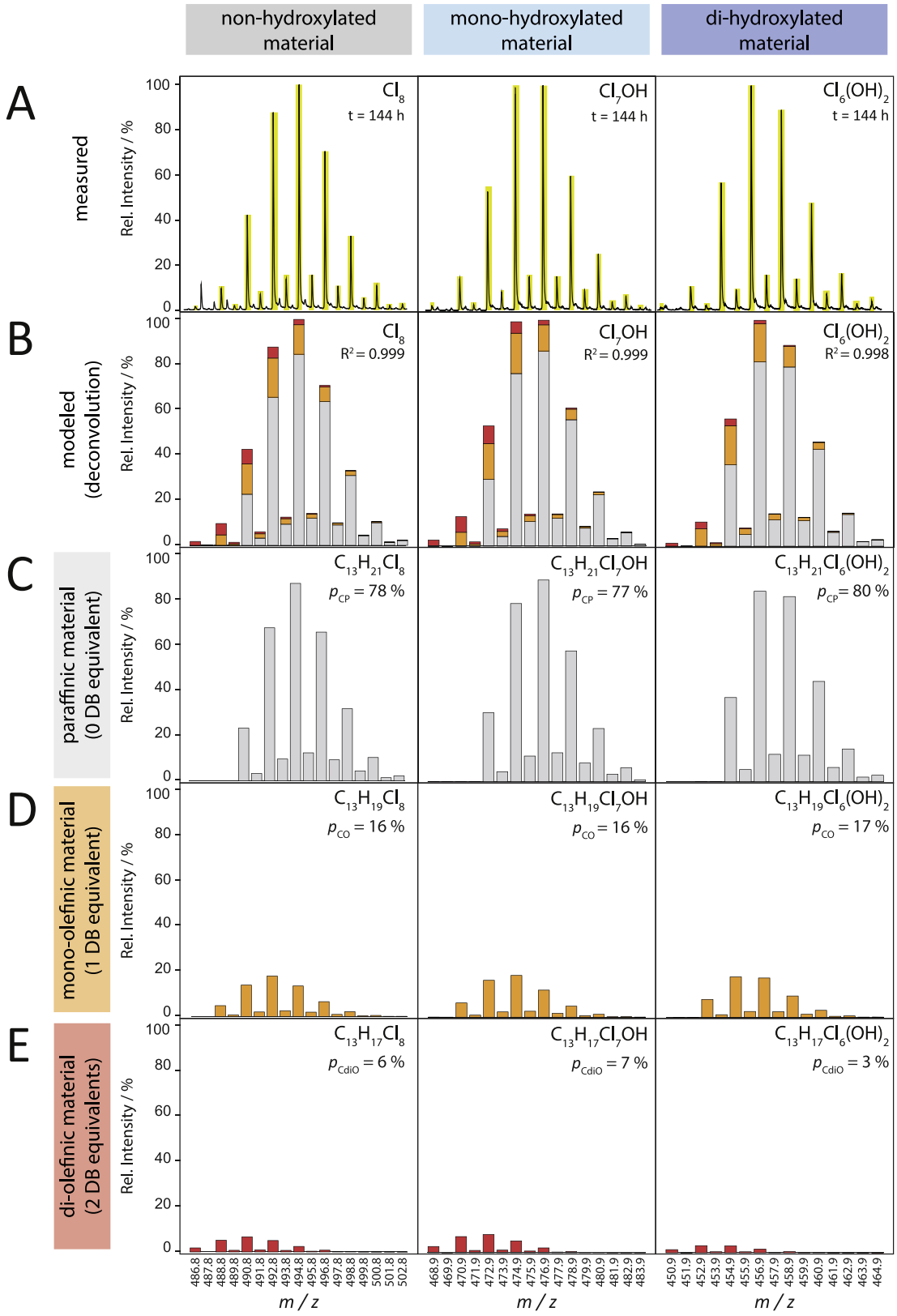


Fig. 5. Mathematical deconvolution of interfered chloride adduct $[M+Cl]^-$ isotope clusters. Mass spectra of non-hydroxylated (Cl_8, left), mono-hydroxylated (Cl_7OH, middle) and di-hydroxylated ($Cl_6(OH)_2$, right) compounds from a LinB-exposed (144 h) chlorotridecane mixture are shown. Highlighted (yellow) are those signals of the measured isotope clusters (A) that were used for mathematical deconvolution. Modeled isotope clusters (B) are compared with measured (A) ones and coefficients of determination (R^2) are reported as indications of the quality of the models. Resulting proportions of paraffinic (C, p_{CP}, gray), mono-olefinic (D, p_{CO}, orange) and di-olefinic (E, p_{CdiO}, red) materials are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

compounds. For example, oxygenated CP transformation products with one double bond-equivalent can be classified as chlorinated olefinols, chlorinated ketones, chlorinated aldehydes or chlorinated cyclic ethers. However, the proposed mechanism of LinB-catalyzed transformations (Fig. 1C) of CPs is expected to result in CPols and CPdiols. Accordingly, we assume an enzymatic formation of chloroolefin-ols and -diols starting with chloroolefins. Therefore, we suppose that unsaturated LinB-metabolites are hydroxylated compounds too. The terms mono-olefinic and di-olefinic are used for transformation products with one or two double bond-equivalents, respectively.

Fig. S12 shows that isotope clusters of mono-oxygenated Cl_6-, Cl_7-, Cl_8-, and Cl_9-CP materials were also detected in the starting material at low signal-to-noise ratios (S/N 3:1 to 5:1). Mathematical deconvolution shows that these isotope clusters contain low proportions of paraffinic material p_{CP} of 14%, 31%, 58% and 77%, respectively. Mean chlorination degree n_{Cl} of these paraffinic oxygenated compounds is 8.10. The control samples (exposed to buffer only, 144 h) show comparable results. Therefore, no indications for an abiotic hydrolysis were found. After LinB-exposure, detected isotope clusters of mono-oxygenated compounds were one to two orders of magnitude (8-78x) more prominent, showed higher paraffinic proportions and lower chlorination degree. Results of the LinB-exposed sample are discussed later in detail. We conclude that traces of mono-oxygenated substances can be found in the starting material but their chemical structure differs from the ones obtained after LinB-exposure. Dihydroxylated compounds were not found in the starting material and the control sample.

3.4. Deconvolution of interfered spectra of CPs and hydroxylated transformation products

3.4.1. Proportions of paraffinic, mono-olefinic and di-olefinic compounds

Non-hydroxylated, mono-hydroxylated and di-hydroxylated chlorinated tridecanes could be separated from each other by liquid chromatography. Therefore, these compounds do not interfere with each other. Nevertheless, mass spectra of said compounds are interfered with signals of mono-olefinic and di-olefinic compounds. The mathematical deconvolution procedure provides paraffinic, mono-olefinic and di-olefinic proportions, which are discussed in this section.

Isotope clusters of hepta- to undeca-chlorotridecanes (Fig. 3A, B), of hexa- to deca-chlorotridecanols (Fig. 3C) and of penta- to nona-chlorotridecanediols (Fig. 3D), were evaluated with the mathematical deconvolution procedure. Tables S2–S4 (SI) show average (n = 2) proportions of paraffinic, mono-olefinic and di-olefinic materials in isotope clusters of non-exposed and LinB-exposed material and respective hydroxylated transformation products. As an example, Fig. 5 displays measured isotope clusters (A) of LinB-exposed octachloro-, heptachloro-hydroxy- and hexachloro-dihydroxy-compounds. The hydroxylated compounds are the expected first- and second-generation products of the octachloro-tridecanes in the mixture. Modeled isotope clusters (Fig. 5B) show signal distributions of interfering paraffinic (gray), mono-olefinic (orange) and di-olefinic (red) material. Paraffinic compounds (Fig. 5C), with fractions of p_{CP} of 78%, 77% and 80%, dominated in the non-, mono- and di-hydroxylated materials. Mono-olefinic (Fig. 5D) and di-olefinic (Fig. 5E) materials contribute with proportions p_{CO} of 16%, 16% and 17% and p_{CdiO} of 6%, 7% and 3%, respectively.

As shown in Table S2 in both, non-exposed and LinB-exposed materials, proportions of paraffinic material p_{CP} increased with chlorine number. In the non-exposed materials, p_{CP} of 67 ± 1%, 83 ± 3%, 89 ± 0%, 94 ± 0% and 95 ± 1% were obtained for Cl_7-, Cl_8-, Cl_9-, Cl_{10}-, and Cl_{11}-homologues. Whereas in the exposed sample p_{CP} of 57 ± 1%, 78 ± 1%, 88 ± 1%, 93 ± 1% and 97 ± 1% were found respectively (Table S2).

Tables S3 and S4 list paraffin-, mono-olefin- and di-olefin-proportions of enzymatically formed mono- and di-hydroxylated transformation products. Paraffinic proportions increased from 47 ± 2% to 77 ± 0%, 88 ± 1%, 94 ± 0% and 93 ± 1% for mono-hydroxylated Cl_6-, Cl_7-, Cl_8-, Cl_9- and Cl_{10}-homologues (Table S3). Accordingly, paraffinic proportions for dihydroxylated Cl_5-, Cl_6-, Cl_7-, Cl_8- and Cl_9-homologues increased from 51 ± 1% to 82 ± 0%, 97 ± 1%, 100 ± 0% and 100 ± 0% (Table S4).

To conclude, paraffinic proportions in all examined isotope clusters increase with chlorination degree.

This trend is observed for mono- and di-hydroxylated transformation products as well as for parent compounds in both, LinB-exposed and non-exposed material.

Overall, it was found that LinB did not affect the proportions of paraffinic, mono-olefinic and di-olefinic compounds, neither in the starting material nor in the hydroxylated transformation products. Any dehydrochlorination activity (loss of HCl) catalyzed by LinB, would result in higher proportions of olefinic material. In addition, the detection of lower chlorinated materials ($Cl_{<4}$), after LinB-exposure, would be expected. For example, pentachloro tridecenes, tetrachlorotridecenols and trichlorotridecenediols would be expected dehydrochlorination products. However, these substances were not detected. In conclusion, LinB catalyzes dehalohydroxylation reactions (S_N2) of CPs, COs and CdiOs, forming hydroxylated transformation products, but does not catalyze dehydrochlorination reactions (E2).

3.4.2. Changes of chlorination degree during LinB exposure

Deconvolution of interfered spectra not only gives proportions of paraffinic, mono-olefinic and di-olefinic material, it also gives non-interfered signal intensities ($I_{100\%}$) as demonstrated in Fig. S1 (SI, step 7). Tables S5–S7 report non-interfered signal intensities ($I_{100\%}$) of non-, mono- and di-hydroxylated materials before and after LinB-exposure. Chlorination (n_{Cl}) and hydroxylation (n_{OH}) degrees were deduced from these data given as average number of chlorine atoms and hydroxy groups, respectively.

Table 1 displays degrees of chlorination n_{Cl} of different materials at start and after exposure to buffer and enzyme. Overall, the starting material and the non-exposed control show comparable chlorination degrees of paraffinic and olefinic material (Table 1). This indicates that observed transformation reactions of the starting material are not induced by buffer but LinB-catalyzed.

During LinB-exposure, the chlorination degrees of CPs and COs increased from 8.80 and 8.37 to 9.07 and 8.55 (Table 1). Chlorination degrees of mono-hydroxylated CPols and COols were found to be 7.59 and 7.04. This corresponds to chlorine losses of 1.21 and 1.33 with respect to the CP and CO precursors. Dihydroxylated transformation products are also detected in the LinB-exposed sample. Chlorination degrees of CPdiols and COdiols are 6.61 and 5.23, corresponding to chlorine losses of 2.19 and 3.32 compared to respective non-hydroxylated starting materials.

With respect to CPs and COs, n_{Cl} values increased during LinB exposure. We conclude that higher chlorinated CPs and COs were transformed slower than lower chlorinated ones. In other words, higher chlorinated material are more stable towards LinB-catalyzed transformation. Differences of n_{Cl} of mono- and di-hydroxylated products with respect to non-hydroxylated precursors are lower for paraffinic than mono-olefinic materials. This effect can be explained by the finding that chlorination degrees of COs in the starting material are already lower than those of CPs.

The mean number of hydroxyl groups attached to families of compounds consisting of non-, mono- and di-hydroxylated

Table 1
Chlorination (n_{Cl}) and hydroxylation (n_{OH}) degrees of non-exposed and LinB-exposed material. Displayed are paraffinic and mono-olefinic compounds. Di-olefinic compounds are not listed because of low abundance, both in the staring material and the transformation products.

		Chlorination degree of related compounds n_{Cl}			Hydroxylation degree of CPs homologues and transformation products n_{OH}					
		n_{Cl} non-exposed (0h)	n_{Cl} exposed (144h)		Starting material	-	1st generation product	-	2nd generation product	n_{OH} exposed
Paraffinic material	Non-hydroxylated	8.80 ± 0.00	9.07 ± 0.00	Paraffinic material	Cl_7	–	$Cl_6(OH)$	–	$Cl_5(OH)_2$	0.68
					Cl_8	–	$Cl_7(OH)$	–	$Cl_6(OH)_2$	0.51
	Mono-hydroxylated	8.31 ± 0.06[a]	7.59 ± 0.01		Cl_9	–	$Cl_8(OH)$	–	$Cl_7(OH)_2$	0.39
	Di-hydroxylated	n.d.	6.61 ± 0.04		Cl_{10}	–	$Cl_9(OH)$	–	$Cl_8(OH)_2$	0.28
					Cl_{11}	–	$Cl_{10}(OH)$	–	$Cl_9(OH)_2$	0.16
Olefinic material	Non-hydroxylated	8.37 ± 0.02	8.55 ± 0.01	Olefinic material	Cl_7	–	$Cl_6(OH)$	–	$Cl_5(OH)_2$	0.86
					Cl_8	–	$Cl_7(OH)$	–	$Cl_6(OH)_2$	0.46
	Mono-hydroxylated	8.31 ± 0.06[a]	7.04 ± 0.03		Cl_9	–	$Cl_8(OH)$	–	$Cl_7(OH)_2$	0.17
	Di-hydroxylated	n.d.	5.23 ± 0.01		Cl_{10}	–	$Cl_9(OH)$	–	$Cl_8(OH)_2$	0.11
					Cl_{11}	–	$Cl_{10}(OH)$	–	$Cl_9(OH)_2$	0.07

n.d. not detected.
[a] Some uncertainties have to be expected due to lower signal-to-noise ratios.

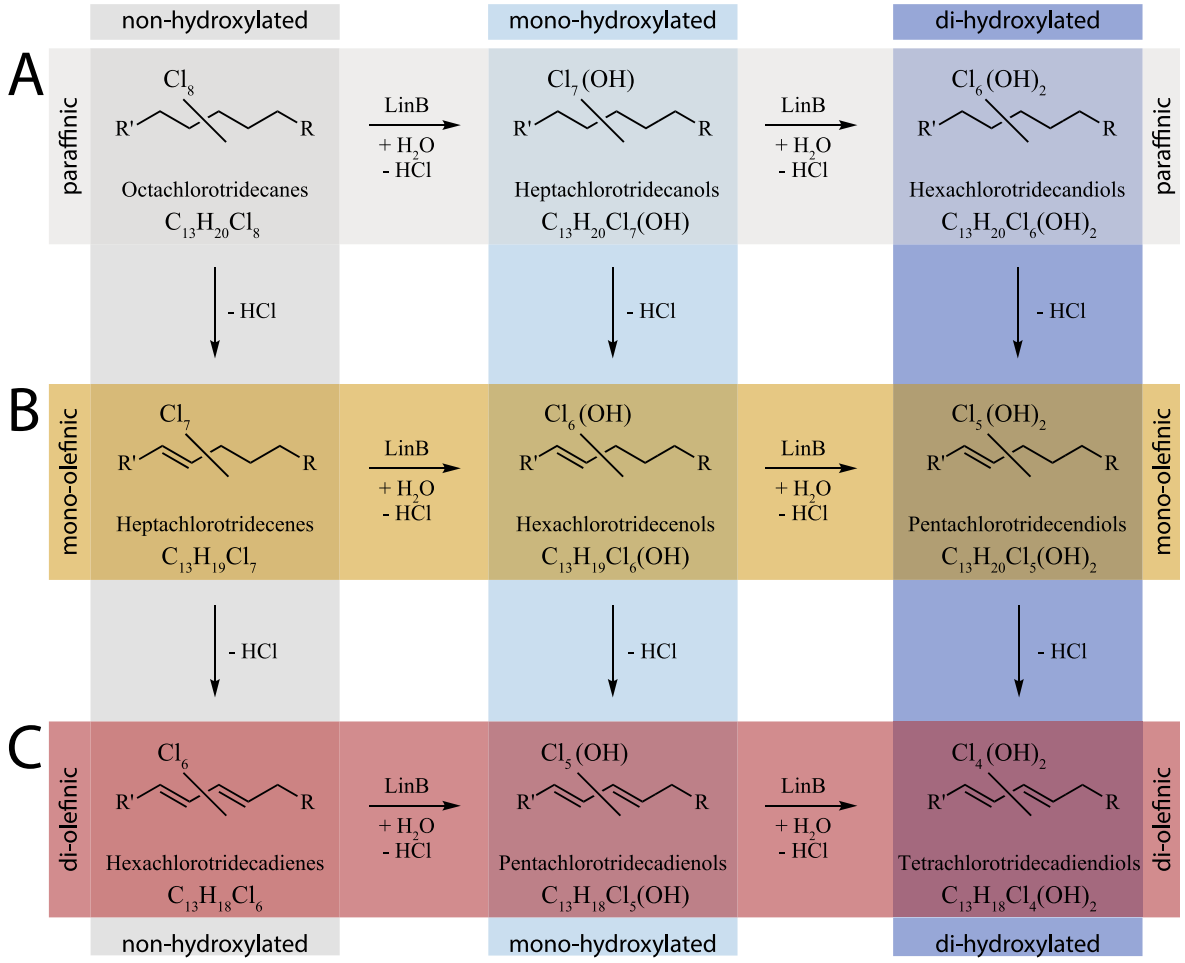


Fig. 6. Reaction-scheme of all detected octachlorodecane transformation products. LinB-catalyzed dehalohydroxylation of octachlorotridecanes (A, gray) results in the formation of heptachlorotridecanols (light blue) and hexachlorotridecanediols (dark blue). Abiotic and biotic elimination reactions (E2) may lead to heptachlorotridecenes (B, orange) and subsequently to hexachlorotridecadienes (C, red). However, LinB-catalyzed elimination reaction of hydrogen chloride were not observed, but dehalohydroxylation of mono-olefinic and di-olefinic material already present in the starting material was catalyzed by LinB. Combinations of such biotic and abiotic elimination- and dehalohydroxylation-reactions result in these complex mixtures of lower chlorinated, saturated and unsaturated, mono- and dihydroxylated transformation products. Accordingly, similar transformation products were obtained for all other Cl_6-, Cl_7-, Cl_8-, Cl_9-, Cl_{10}- and Cl_{11}-homologues. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

materials was calculated based on $I_{100\%}$-signals and is reported in Table 1. Respective hydroxylation degrees (n_{OH}) for Cl_7- to Cl_{11}-precursors increased during LinB exposure to 0.68, 0.51, 0.39, 0.28 and 0.16 for paraffinic materials. Hydroxylation degrees of 0.86, 0.46, 0.17, 0.11 and 0.07 were obtained for mono-olefinic material. We conclude that hydroxylation degrees are higher for lower chlorinated precursors than for higher chlorinated ones. This indicates again that LinB preferentially transforms lower chlorinated CPs and COs.

Overall, we conclude that LinB dehalohydroxylates CPs and COs stepwise to mono- and di-hydroxylated transformation products. The LinB-catalyzed transformation of lower chlorinated CPs and COs is preferred and higher chlorinated material is more persistent and is therefore accumulated in relative amounts in the remaining starting material.

3.5. Biotransformation of SCCPs with LinB leads to complex mixtures of metabolites

This study could show that LinB catalyzes dehalohydroxylation (S_N2) reactions of materials consisting mainly of short-chain CPs, but also of COs and CdiOs, to mono- and dihydroxlated compounds. Elimination reactions (E2) of hydrogen chloride of SCCPs to mono-olefinic and di-olefinic transformation products were not induced by LinB. However, in combination with other biotic and abiotic dehydrochlorination and oxidation pathways mono-olefinic and di-olefinic compounds with variable hydroxylation degree can also be formed (Liu et al., 2015; Schinkel et al., 2018a; Heeb et al., 2019). This leads to complex mixtures of related compounds with variable degrees of saturation, chlorination and hydroxylation. Fig. 6 shows a scheme of potential biotic and abiotic transformation reactions starting from octachlorotridecanes as an example. The proposed elimination (E2) and substitution reactions (S_N2) lead to paraffinic (A), mono-olefinic (B), di-olefinic (C) as well as non-, mono- and di-hydroxylated transformation products. SCCPs as such are already mixtures of thousands of congeners with a vast diversity regarding chlorination degree, constitution and stereochemistry. Transformation reactions such as those observed result in even more diverse mixtures.

4. Conclusion

The mass spectrometric analysis of mixtures of chlorinated paraffins, mono-olefins and di-olefins together with their mono- and di-hydroxylated transformation products is challenging. Mass spectra of these complex mixtures are strongly interfered. It has been demonstrated that paraffinic, mono-olefinic and di-olefinic compounds cannot be distinguished at a mass resolution <10′000. However, after mathematical deconvolution, these interferences could be resolved and the individual fractions of paraffins, mono-olefins and di-olefins could be assessed. Furthermore, evidence was presented that the LinB-enzyme *in vitro* catalyzes substitution reactions (S_N2) of chlorine atoms with hydroxy groups of certain SCCPs and with it the formation of mono- and di-hydroxylated products.

Accordingly, some of the mono-olefinic materials were transformed to mono- and di-hydroxylated chloroolefins as well. Chlorodiolefins, present in small amounts in the starting material, were also converted by LinB to respective mono- and di-hydroxylated products. It was found that LinB did not catalyze elimination (E2) of HCl of paraffinic to olefinic substances.

Mono- and di-hydroxylated products were separated from the starting material on a C18-reverse phase LC-column. Sets of retention time indices were established for these newly formed hydroxylated metabolites. Liquid chromatographic separation of non-hydroxylated material and hydroxylated transformation products was needed to prevent additional interferences.

Chlorination degrees of the remaining material increased from 8.80 to 9.07 during LinB-exposure. Chlorination degrees of mono- and di-hydroxylated products were 7.59 and 6.61. Thus, the *in vitro* transformation with the LinB-enzyme led to chlorine losses of 1.21 and 2.19. Hence, CPs were both, dehalogenated and hydroxylated. Mainly lower chlorinated alcohols ($Cl_{<8}$) were formed and higher chlorinated compounds ($Cl_{>9}$) accumulated in relative amounts. This suggests that higher chlorinated CP homologues might be more persistent than lower chlorinated ones and eventually will accumulate in environments when alike bacterial transformation may occur. With the presented methods, effects of CP carbon chain lengths regarding enzyme-catalyzed dehalohydroxylation reactions can be investigated in further studies.

To the best of our knowledge, this is the first example of an enzyme-catalyzed dehalohydroxylation reaction of SCCPs. The mono- and di-hydroxylated products formed eluted in more polar conditions than the starting material. Most likely, these hydroxylated CPs are therefore better water soluble than their parent CPs.

The presented findings show that hydroxylated CPs indeed are transformation products that may form in bacteria. Such metabolites are likely to be present in the aquatic environment. However, the environmental and toxicological impact of such hydroxylated CP transformation products are yet unknown and need to be further investigated.

Credit author statement

Marco Knobloch: Methodology, Validation, Software, Formal analysis, Investigation, Visualization, Writing - Original Draft, Review & Editing. Lena Schinkel: Methodology, Software, Writing - Review & Editing. Hans-Peter Kohler: Conceptualization, Supervision, Writing - Review & Editing. Iris Schilling: Methodology, Investigation. Peter Lienemann: Conceptualization, Project administration. Davide Bleiner: Supervision, Project administration. Norbert Heeb: Conceptualization, Methodology, Validation, Project administration, Funding acquisition, Supervision, Visualization, Writing - Review & Editing.

Funding

This work was supported by the Swiss Federal Office for the Environment (BAFU) (grant number: 19.0011.PJ/S113-1600).

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.

Acknowledgement

We thank Thomas Fleischmann (Eawag) for his help and assistance in obtaining the LinB-enzyme.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128288.

References

Bogdal, C., Alsberg, T., Diefenbacher, P.S., Macleod, M., Berger, U., 2015. Fast

quantification of chlorinated paraffins in environmental samples by direct injection high-resolution mass spectrometry with pattern deconvolution. Anal. Chem. 87, 2852–2860. https://doi.org/10.1021/ac504444d.
Chen, W., Yu, M., Zhang, Q., Hou, X., Kong, W., Wei, L., Mao, X., Liu, J., Schnoor, J.L., Jiang, G., 2020. Metabolism of SCCPs and MCCPs in Suspension Rice Cells Based on Paired Mass Distance (PMD) Analysis. https://doi.org/10.1021/acs.est.0c01830.
Copley, S.D., 1999. Microbial dehalogenases. Compr. Nat. Prod. Chem. 401–422. https://doi.org/10.1016/b978-0-08-091283-7.00122-3.
de Wit, C.A., Bossi, R., Dietz, R., Dreyer, A., Faxneld, S., Garbus, S.E., Hellström, P., Koschorreck, J., Lohmann, N., Roos, A., Sellström, U., Sonne, C., Treu, G., Vorkamp, K., Yuan, B., Eulaers, I., 2020. Organohalogen compounds of emerging concern in Baltic Sea biota: levels, biomagnification potential and comparisons with legacy contaminants. Environ. Int. 144, 106037. https://doi.org/10.1016/j.envint.2020.106037.
ECHA, 2019. Substance Evaluation Conclusion for Medium-Chain Chlorinated Paraffins/alkanes, C14-17, Chloro. https://echa.europa.eu/de/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e3841.
Fiedler, H., 2010. Short-chain chlorinated paraffins: production, use and international regulations. In: The Handbook of Environmental Chemistry - Chlorinated Paraffins. Springer Nature, pp. 1–40. https://doi.org/10.1007/698.
Glüge, J., Wang, Z., Bogdal, C., Scheringer, M., Hungerbühler, K., 2016. Global production, use, and emission volumes of short-chain chlorinated paraffins – a minimum scenario. Sci. Total Environ. 573, 1132–1146. https://doi.org/10.1016/j.scitotenv.2016.08.105.
Glüge, J., Schinkel, L., Hungerbühler, K., Cariou, R., Bogdal, C., 2018. Environmental risks of medium-chain chlorinated paraffins (MCCPs): a review. Environ. Sci. Technol. 52, 6743–6760. https://doi.org/10.1021/acs.est.7b06459.
Heeb, N.V., Zindel, D., Graf, H., Azara, V., Bernd Schweizer, W., Geueke, B., Kohler, H.P.E., Lienemann, P., 2013. Stereochemistry of LinB-catalyzed biotransformation of δ-HBCD to 1R,2R,5S,6R,9R,10S-pentabromocyclododecanol. Chemosphere 90, 1911–1919. https://doi.org/10.1016/j.chemosphere.2012.10.019.
Heeb, N.V., Wyss, S.A., Geueke, B., Fleischmann, T., Kohler, H.P.E., Lienemann, P., 2014. LinA2, a HCH-converting bacterial enzyme that dehydrohalogenates HBCDs. Chemosphere 107, 194–202. https://doi.org/10.1016/j.chemosphere.2013.12.035.
Heeb, N.V., Grubelnik, A., Geueke, B., Kohler, H.P.E., Lienemann, P., 2017. Biotransformation of hexabromocyclododecanes with hexachlorocyclohexane-transforming Sphingobium chinhatense strain IP26. Chemosphere 182, 491–500. https://doi.org/10.1016/j.chemosphere.2017.05.047.
Heeb, N.V., Schalles, S., Lehner, S., Schinkel, L., Schilling, I., Lienemann, P., Bogdal, C., Kohler, H.P.E., 2019. Biotransformation of short-chain chlorinated paraffins (SCCPs) with LinA2: a HCH and HBCD converting bacterial dehydrohalogenase. Chemosphere 226, 744–754. https://doi.org/10.1016/j.chemosphere.2019.03.169.
Hirschorn, S.K., Grostern, A., Lacrampe-Couloume, G., Edwards, E.A., MacKinnon, L., Repta, C., Major, D.W., Sherwood Lollar, B., 2007. Quantification of biotransformation of chlorinated hydrocarbons in a biostimulation study: added value via stable carbon isotope analysis. J. Contam. Hydrol. 94, 249–260. https://doi.org/10.1016/j.jconhyd.2007.07.001.
Iozza, S., Schmid, P., Oehme, M., Bassan, R., Belis, C., Jakobi, G., Kirchner, M., Schramm, K.-W., Kräuchi, N., Moche, W., Offenthaler, I., Weiss, P., Simoncic, P., Knoth, W., 2009a. Altitude profiles of total chlorinated paraffins in humus and spruce needles from the Alps (MONARPOP). Environ. Pollut. 157, 3225–3231. https://doi.org/10.1016/j.envpol.2009.05.033.
Iozza, S., Schmid, P., Oehme, M., 2009b. Development of a comprehensive analytical method for the determination of chlorinated paraffins in spruce needles applied in passive air sampling. https://doi.org/10.1016/j.envpol.2009.06.033, 157, 3218, 3224.
Korytár, P., Parera, J., Leonards, P.E.G., Santos, F.J., De Boer, J., Brinkman, U.A.T., 2005. Characterization of polychlorinated n-alkanes using comprehensive two-dimensional gas chromatography-electron-capture negative ionisation time-of-flight mass spectrometry. J. Chromatogr. A 1086, 71–82. https://doi.org/10.1016/j.chroma.2005.05.003.
Lal, R., Dogra, C., Malhotra, S., Sharma, P., Pal, R., 2006. Diversity, distribution and divergence of lin genes in hexachlorocyclohexane-degrading sphingomonads. Trends Biotechnol. 24, 121–130. https://doi.org/10.1016/j.tibtech.2006.01.005.
Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H.-P.E., Holliger, C., Jackson, C., Oakeshott, J.G., 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol. Mol. Biol. Rev. 74, 58–80. https://doi.org/10.1128/MMBR.00029-09.
Li, H., Fu, J., Zhang, A., Zhang, Q., Wang, Y., 2016. Occurrence, bioaccumulation and long-range transport of short-chain chlorinated paraffins on the Fildes Peninsula at King George Island, Antarctica. Environ. Int. 94, 408–414. https://doi.org/10.1016/j.envint.2016.05.005.
Liu, R., Zhang, C., Kang, L., Sun, X., Zhao, Y., 2015. The OH-initiated chemical transformation of 1,2,4,6,8,10,11-heptachloroundecane in the atmosphere. RSC Adv. 5, 37988–37994. https://doi.org/10.1039/C5RA00612K.
Liu, L.L., Li, Y., Coelhan, M., Chan, H.M., Ma, W., Liu, L.L., Man, H., Ma, W., Liu, L.L., 2016. Relative developmental toxicity of short-chain chlorinated paraffins in Zebrafish (Danio rerio) embryos. Environ. Pollut. 219, 1122–1130. https://doi.org/10.1016/j.envpol.2016.09.016.
Loos, M., Gerber, C., Corona, F., Hollender, J., Singer, H., 2015. Accelerated isotope fine structure calculation using pruned transition trees. Anal. Chem. 87, 5738–5744. https://doi.org/10.1021/acs.analchem.5b00941.
Manna, R.N., Dybala-Defratyka, A., 2014. A computational study of the dechlorination of β-hexachlorocyclohexane (β-HCH) catalyzed by the haloalkane dehalogenase LinB. Arch. Biochem. Biophys. 562, 43–50. https://doi.org/10.1016/j.abb.2014.07.028.
Müller, R., Lingens, F., 1986. Microbial degradation of halogenated hydrocarbons: a biological solution to pollution problems? Angew Chem. Int. Ed. Engl. 25, 779–789. https://doi.org/10.1002/anie.198607791.
Nagata, Y., Miyauchi, K., Takagi, M., 1999. Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in Sphingomonas paucimobilis UT26. J. Ind. Microbiol. Biotechnol. 23, 380–390. https://doi.org/10.1038/sj.jim.2900736.
Oakley, A.J., Prokop, Z., Boháč, M., Kmuníček, J., Jedlička, T., Monincová, M., Kutá-Smatanová, I., Nagata, Y., Damborský, J., Wilce, M.C.J., 2002. Exploring the structure and activity of haloalkane dehalogenase from Sphingomonas paucimobilis UT26: evidence for product- and water-mediated inhibition. Biochemistry 41, 4847–4855. https://doi.org/10.1021/bi015734i.
Pal, R., Bala, S., Dadhwal, M., Kumar, M., Dhingra, G., Prakash, O., Prabagaran, S.R., Shivaji, S., Cullum, J., Holliger, C., Lal, R., 2005. Hexachlorocyclohexane-degrading bacterial strains Sphingomonas paucimobilis B90A. UT26 and Sp+, having similar lin genes, represent three distinct species, Sphingobium indicum sp. nov., Sphingobium japonicum sp. nov. and Sphingobium francense sp. nov., an. Int. J. Syst. Evol. Microbiol. 55, 1965–1972. https://doi.org/10.1099/ijs.0.63201-0.
Perkons, I., Pasecnaja, E., Zacs, D., 2019. The impact of baking on chlorinated paraffins: characterization of C10–C17 chlorinated paraffins in oven-baked pastry products and unprocessed pastry dough by HPLC–ESI–Q–TOF–MS. Food Chem. 298, 125100. https://doi.org/10.1016/j.foodchem.2019.125100.
Prokop, Z., Monincová, M., Chaloupková, R., Klvaňa, M., Nagata, Y., Janssen, D.B., Damborský, J., 2003. Catalytic mechanism of the haloalkane dehalogenase LinB from Sphingomonas paucimobilis UT26. J. Biol. Chem. 278, 45094–45100. https://doi.org/10.1074/jbc.M307056200.
Raina, V., Hauser, A., Buser, H.R., Rentsch, D., Sharma, P., Lal, R., Holliger, C., Poiger, T., Müller, M.D., Kohler, H.P.E., 2007. Hydroxylated metabolites of β- and δ-hexachlorocyclohexane: bacterial formation, stereochemical configuration, and occurrence in groundwater at a former production site. Environ. Sci. Technol. 41, 4291–4298. https://doi.org/10.1021/es062908g.
Schinkel, L., Lehner, S., Heeb, N.V., Lienemann, P., McNeill, K., Bogdal, C., 2017. Deconvolution of mass spectral interferences of chlorinated alkanes and their thermal degradation products: chlorinated alkenes. Anal. Chem. 89, 5923–5931. https://doi.org/10.1021/acs.analchem.7b00331.
Schinkel, L., Lehner, S., Knobloch, M., Lienemann, P., Bogdal, C., McNeill, K., Heeb, N.V., 2018a. Transformation of chlorinated paraffins to olefins during metal work and thermal exposure – deconvolution of mass spectra and kinetics. Chemosphere 194. https://doi.org/10.1016/j.chemosphere.2017.11.168.
Schinkel, L., Bogdal, C., Canonica, E., Cariou, R., Bleiner, D., McNeill, K., Heeb, N.V., 2018b. Analysis of medium-chain and long-chain chlorinated paraffins: the urgent need for more specific analytical standards. Environ. Sci. Technol. Lett. 5, 708–717. https://doi.org/10.1021/acs.estlett.8b00537.
Schinkel, L., Lehner, S., Heeb, N.V., Marchand, P., Cariou, R., McNeill, K., Bogdal, C., 2018c. Dealing with strong mass interferences of chlorinated paraffins and their transformation products: an analytical guide. TrAC Trends Anal. Chem. (Reference Ed.) 106, 116–124. https://doi.org/10.1016/j.trac.2018.07.002.
Senoo, K., Wada, H., 1989. Isolation and identification of an aerobic γ-HCH-decomposing bacterium from soil. Soil Sci. Plant Nutr. 35, 79–87. https://doi.org/10.1080/00380768.1989.10434739.
Streltsov, V.A., Prokop, Z., Damborský, J., Nagata, Y., Oakley, A., Wilce, M.C.J., 2003. Haloalkane dehalogenase LinB from Sphingomonas paucimobilis UT26: X-ray crystallographic studies of dehalogenation of brominated substrates. Biochemistry 42, 10104–10112. https://doi.org/10.1021/bi027280a.
Tomy, G.T., 2009. Analysis of Chlorinated Paraffins in Environmental Matrices: the Ultimate Challenge for the Analytical Chemist, pp. 83–106. https://doi.org/10.1007/698_2009_39.
UNEP, 2009. UNEP/POPS/COP.4/38 Report of the Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants on the Work of its Fourth Meeting, vol. 11, pp. 329–342.
UNEP, 2016. Report of the Persistent Organic Pollutants Review Committee on the Work of its Twelfth Meeting. UNEP/POPS/POPRC.12/11/Add.3 1–36.
UNEP, 2017. Eighth Meeting of the Conference of the Parties to the Stockholm Convention. UNEP/POPS/COP.8/32.
van Mourik, L.M., Gaus, C., Leonards, P.E.G., de Boer, J., 2016. Chlorinated paraffins in the environment: a review on their production, fate, levels and trends between 2010 and 2015. Chemosphere 155, 415–428. https://doi.org/10.1016/j.chemosphere.2016.04.037.
van Mourik, L.M., van der Veen, I., Crum, S., de Boer, J., 2018. Developments and interlaboratory study of the analysis of short-chain chlorinated paraffins. TrAC Trends Anal. Chem. (Reference Ed.) 102, 32–40. https://doi.org/10.1016/j.trac.2018.01.004.
Vijgen, J., Abhilash, P.C., Li, Y.F., Lal, R., Forter, M., Torres, J., Singh, N., Yunus, M., Tian, C., Schäffer, A., Weber, R., 2011. Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs-a global perspective on the management of Lindane and its waste isomers. Environ. Sci. Pollut. Res. 18, 152–162. https://doi.org/10.1007/s11356-010-0417-9.

Vogel, T.M., Criddle, C.S., McCarty, P.L., Arcos, J.C., 1987. Transformations of halogenated aliphatic compounds: oxidation, reduction, substitution, and dehydrohalogenation reactions occur abiotically or in microbial and mammalian systems. Environ. Sci. Technol. 21, 722–736. https://doi.org/10.1021/es00162a001.

Vorkamp, K., Rigét, F.F., 2014. A review of new and current-use contaminants in the Arctic environment: evidence of long-range transport and indications of bioaccumulation. Chemosphere 111, 379–395. https://doi.org/10.1016/j.chemosphere.2014.04.019.

Willett, K.L., Ulrich, E.M., Hites, R.A., 1998. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ. Sci. Technol. 32, 2197–2207. https://doi.org/10.1021/es9708530.

Yuan, B., Alsberg, T., Bogdal, C., MacLeod, M., Berger, U., Gao, W., Wang, Y., De Wit, C.A., 2016. Deconvolution of soft ionization mass spectra of chlorinated paraffins to resolve congener groups. Anal. Chem. 88, 8980–8988. https://doi.org/10.1021/acs.analchem.6b01172.

Yuan, B., Vorkamp, K., Roos, A.M., Faxneld, S., Sonne, C., Garbus, S.E., Lind, Y., Eulaers, I., Hellström, P., Dietz, R., Persson, S., Bossi, R., de Wit, C.A., 2019. Accumulation of short-, medium-, and long-chain chlorinated paraffins in marine and terrestrial animals from scandinavia. Environ. Sci. Technol. 53, 3526–3537. https://doi.org/10.1021/acs.est.8b06518.

Zencak, Z., Reth, M., Oehme, M., 2003. Dichloromethane-enhanced negative ion chemical ionization for the determination of polychlorinated n-alkanes. Anal. Chem. 75, 2487–2492. https://doi.org/10.1021/ac034090c.

Zeng, L., Lam, J.C.W., Wang, Y., Jiang, G., Lam, P.K.S., 2015. Temporal trends and pattern changes of short- and medium-chain chlorinated paraffins in marine mammals from the south China sea over the past decade. Environ. Sci. Technol. 49, 11348–11355. https://doi.org/10.1021/acs.est.5b02473.