Biogeochemistry 62: 277–287, 2003.

De novo formation of organochlorines in a sewage treatment plant

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Received 19 February 2001; accepted in revised form 14 February 2002

Key words: AOX, Dichlorophenylacetic acid, Naturally formed organohalogens, Slaughterhouse

Abstract. The de novo formation of organochlorines was observed in a municipal sewage treatment plant. Due to this formation, the amount of organically bound halogens (AOX) increased 15-fold inside the sewage treatment plant. Per day, more than 6 kg of organically-bound chlorine were produced. This formation is not based on a metabolism of present organochlorines, it is a de novo formation out of inorganic chloride and organic substrates. The AOX trigger concentration in sewage sludge in Germany is 500 mg kg^{-1} and was sometimes exceeded by a factor of 10. No known anthropogenic organohalogens were found which could explain the elevated AOX concentrations. Instead many chlorinated compounds could be identified which were not known to be of anthropogenic origin. The compound with the highest concentration was the 3,4-dichlorophenylacetic acid (3,4-CPAc). In one case, more than 1 g kg^{-1} of this compound was detected. A slaughterhouse that emits phenylacetic acid is probably the origin of that formation. In model experiments phenylacetic acid was chlorinated with HOCl but chlorinated phenylacetic acids other than 3,4-CPAc were found. Therefore it can be excluded that the chlorination in the sewage treatment plant takes place by an abiotic reaction with hypochlorite that might have been introduced there. We assume that the occurring microorganisms are responsible for the de novo formation in the sewage treatment plant. The obtained knowledge could also be useful to understand natural chlorination processes.

Introduction

Large anthropogenic sources of organohalogens to the environment have existed since the 1950's but only recently has it been revealed that organohalogens are produced naturally as well (Gribble (1996, 1998); Winterton 2000). Natural organohalogens are mostly derived from biotic sources like microorganisms, fungi, enzymes etc., but there are also abiotic reactions that form natural organohalogens (Neidleman and Geigert 1986; Keppler et al. 2000). The natural formation of organohalogens occurs on large scale, e.g. it is estimated that 50% of the organically bound halogens in the river Rhine originate from biogenic sources (Hoekstra and de Leer 1993).

In 1998 it was reported by Gribble that 2,900 naturally-formed halogenated compounds exist, and the number is still rising. Uses for some of these halogenated compounds have been found, such as with the chlorinated antibiotic Chloramphenicol.

The occurrence of natural organohalogens has to be studied to understand the chlorine cycle in the environment. By investigating samples from remote areas in Scandinavia it was found that the background concentration for organically bound halogens is between 0.1 and 5 mg g^{-1} d.w. (Asplund and Grimvall 1991). A natural background of organohalogens was also identified in preindustrial sediment samples (Nkusi and Müller 1995). Natural organohalogens are mainly bound to a fraction > 1000 Dalton (Hjelm and Asplund 1995), therefore it is helpful to use the analytical AOX method, which is a group parameter that allows one to determine the total amount of organically bound halogens. It has been introduced into analytical chemistry by Kühn and Sontheimer (1973) as a general method to detect anthropogenic organohalogens. Therefore restrictions are placed on the discharge of AOX. Nowadays the AOX is also a common tool to detect the natural halogen content of samples from remote areas (Asplund and Grimvall 1991).

In this paper, samples from a sewage treatment plant were studied for formation of organochlorines and discussed in the context of natural chlorination processes.

Materials and methods

Data of the investigated sewage treatment plant

The investigated sewage treatment plant is situated in the northern part of Germany. The treatment plant consists of a biological phosphate elimination, a denitrification unit, a main activation basin and a past cleaning, where the sludge is removed. This treatment plant was planned for 80,000 inhabitants and its working capacity is 90% on average. The daily influent is 3,800 m^3, one fourth of which originates from a large slaughterhouse. Four tons of dried sludge are produced in the sewage treatment plant per day.

Analysis

The AOX of the pressed sludge from the treatment plant was determined periodically over the course of two years (see Figure 1). From December 1996 to June 1998 samples from the activation basin were analyzed for extractable organic compounds by GC/MS. One sample was analyzed after an oxidative degradation method according to Dahlmann et al. (1993) and measured by GC/MS and GC/AED. Wastewater emitted from the slaughterhouse was also analyzed by GC/MS.

The sewage samples were analyzed for AOX according to the standard procedure for sewage sludge (German Standard Methods, DIN (1985)). In short, a sample was diluted with milli-Q water in an Erlenmeyer flask. Activated carbon and acidified nitrate solution were added to the flask and the suspension was shaken for 1 h. The suspension was filtered through a polycarbonate filter and rinsed with an acidic

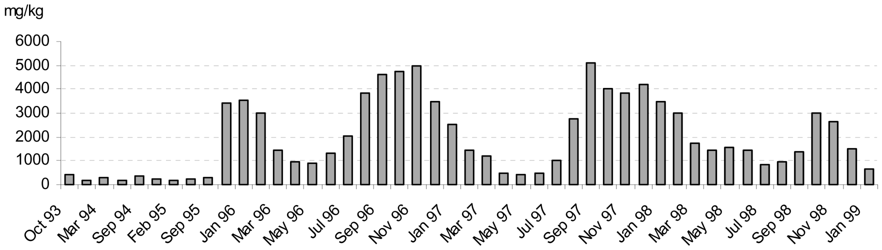


Figure 1. AOX in the sewage sludge in mg kg^{-1} dryweight.

nitrate solution. The filter with the filter cake were then combusted under a stream of oxygen at 1000 °C. The resulting hydrohalides were transferred to a titration cell where they were titrated microcoulometrically. A distinction between organochloride, -bromide and -iodide is not possible with this method. Organofluorides cannot be detected. Additionally NaCl was added to the samples to test if inorganic chloride was removed sufficiently by nitrate washing.

To extract organic compounds, 100 mL of the sample was acidified with concentrated H_2SO_4 to pH 1, 30 mL ethyl acetate was added and it was first ultrasonicated for 15 min and then extracted in a separatory funnel. After saturation with NaCl it was centrifuged for phase separation and again extracted twice in a separatory funnel with 30 mL of ethyl acetate. The organic phases were combined, dried over anhydrous Na_2SO_4 (including 5% $AgNO_3$) and reduced to 1 mL in a graduated pear shape flask. Before measuring the samples by GC/MS, the extract had to be diluted 100 to 10,000 fold with methanol. 2-bromobenzoic acid was used as injection standard.

The diluted extract was derivatized with 50 μL of a 0.2 M methanolic trimethylsulfonium hydroxide solution and measured by GC/MS to determine aromatic acids. Samples that were analyzed for aldehydes and ketones were not derivatized. The GC/MS parameter are: GC: Varian GC 3300, PTV (Carlo Erba MFA 815), 8 μL, carrier gas helium, 120 kPa, columns DB-5MSITD, 30 m, 0.25 mm ID, 0.25 μm, and DB-XLBITD, 30 m, 0.25 mm ID, 0.25 μm, (both J&W Scientific); temperature program: 60 °C (4 min), with 20 °C min^{-1} to 110 °C, with 5 °C min^{-1} to 220 °C, with 20 °C min^{-1} to 280 °C (15 min); MS: Finnigan MAT, Magnum (ion trap), EI 70 eV, full scan modus.

To determine chlorinated acetic acids, the obtained extracts were derivatized with diazomethane and measured by GC/ECD. The GC/ECD parameter are: GC: Fisons HRGC 8265, splitless, 1 μL, carrier gas nitrogen, 130 kPa, column BPX5, 75 m, 0.22 mm ID, 1.0 μm (SGE, Weiterstadt, Germany), temperature program: 50 °C (2 min), with 5 °C min^{-1} to 160 °C (20 min); ECD: Carlo-Erba HT25, ^{63}Ni, 300 °C, make-up gas argon/methane (95/5).

To verify the results obtained from interpretation of the GC/MS mass-spectra, all tentatively identified compounds were purchased or synthesized, if not commercially available, and their GC/MS mass-spectra and GC retention times compared to sample mass-spectra and retention time. The samples were examined for all mono-, di- and trichlorinated benzoic acids (except 2,3,4-CBA); mono- and dichlorinated benzaldehydes; mono- to pentachlorinated phenols; mono- and dichlorinated acetophenones (except 3,5-dichloroacetophenone); monochlorinated phenylacetic acids and -benzonitriles; 2,4-, 2,6- and 3,4-dichlorophenylacetic acid; 2,6- and 3,4-dichlorobenzonitrile as well as mono-, di- and trichloroacetic acid. To exclude a mismatch of the isomers, two differently coated GC-columns were used.

Model experiments with HOCl

Model experiments with HOCl were conducted to determine the ease with which phenylacetic acid can be chlorinated in an aqueous solution and to find out if 3,4-CPAc is the main product of this abiotic chlorination.

Ten microliters of a solution containing 1.000 ppm of phenylacetic acid (PAc) was added in a measuring flask to 3 mL of a 0.1 molar KH_2PO_4-solution (pH 3). To this solution 50 μL of a 15% NaOCl-solution was added. After the reaction time of one hour, the solution was acidified to pH 2 with H_2SO_4 and extracted three times with 5 mL diethyl ether. The organic phases were combined, dried over anhydrous Na_2SO_4, reduced with a rotary evaporator to 1 mL and derivatised with 2 mL of a 0.2 molar diazomethane solution and calibrated with a stream of nitrogen gas to 0.5 mL. This experiment was also repeated with a five fold amount of hypochlorite.

Chemicals

Mono and dichlorobenzoic acids (CBA), 2,3,5- and 2,4,6-CBA as well as chlorinated benzaldehydes (Aldrich, Steinheim, Germany), chlorinated phenols (Supelco, Deisenhofen, Germany), chlorinated acetophenones, – benzonitriles and -phenylacetic acids (Lancaster, Mühlheim am Main, Germany) were used as received. 2,3,6-CBA was purchased from Ehrenstorfer (Augsburg, Germany), while 2,4,5- and 3,4,5-CBA were synthesized. 2,4,5-CBA was produced by oxidation of 2,4,5-trichlorobenzaldehyde according to Brimelow et al. (1951). The corresponding aldehyde was synthesized from 2,4,5-trichloroaniline (Werbel and Elslager 1977) (Lancaster). 3,4,5-CBA was synthesized via a Sandmeyer-reaction starting from 4-amino-3,5-dichlorobenzoic acid (Becker 1990) (Lancaster). Mono- (MCA), di- (DCA) and trichloroacetic acid (TCA) (Merck, Darmstadt, Germany). 2-Bromobenzoic acid (used as an injection standard) was purchased from Aldrich.

Results

Sewage treatment plant samples

Prior to 1995, the sewage sludge of the sewage treatment plant had rather low AOX-values (200 to 400 mg kg^{-1}), which increased suddenly to levels as high as

3.500 mg kg^{-1} (which is 7 times more than the trigger concentration) in 1995 (Figure 1). After this increase, a cyclic behaviour (Figure 1) was observed, with AOX rising to levels as high as 5,100 mg kg^{-1} and as low as 400 mg kg^{-1}.

To elucidate the cause of the high AOX concentrations, the sludge was analyzed for known anthropogenic halogenated compounds such as PCB, PCDD/F, chloropesticides and PVC-dust by a commercial analytical lab according to standard procedures. This analysis found nothing that could explain the high AOX in the sewage sludge either. In a three-week sampling campaign, the AOX of the influent to the plant was continuously measured, but no point contamination was found which could explain the high AOX values. From the results obtained during this sampling campaign, it was found that organically bound organohalogens were introduced into the sewage treatment plant at a rate of 460 g d^{-1} from the daily input of 3,800 m^3 of wastewater with an AOX of 120 μg L^{-1}. 6,160 g d^{-1} AOX were emitted from the plant through the daily production of 4 tons of dried sludge (with a mean AOX of 1,540 mg kg^{-1}). 650 g d^{-1} were released by the effluent with a mean AOX of 170 μg L^{-1}. Therefore, the total amount of emitted organically bound halogens was 6,810 g d^{-1}. These observations indicate that there was a 15-fold increase in AOX within the sewage treatment plant during this sampling campaign.

Analysis of the activated sludge by GC/AED (gas chromatograph with an atomic emission detector) showed that there were many chlorinated compounds but no other AOX-relevant organohalogens (bromine and iodine) present (Figure 2).

By interpreting the mass spectra obtained by GC/MS and controlling the GC retention times with standard compounds, many polar chlorinated aromatic substances were identified. The compound with the highest concentration found was 3,4-dichlorophenylacetic acid (3,4-CPAc) (for GC/MS chromatogram see Figure 3). The highest detected concentration was 1,090 mg kg^{-1}; which accounted for 13% of total AOX in this case (see Figure 4), but this was an anomalously high percentage. This 3,4-dichloro pattern was present in other identified compounds also (see Table 1 for concentrations of the identified compounds from three sampling campaigns, one in December '96 and two in July '97). In addition, mono- and dichloroacetic acid (MCA and DCA), 2,4-dichlorophenol and 3,4,5-trichlorobenzoic acid (CBA) were also detected in some of the samples.

In the samples from March '98 chlorinated acetic acids were also investigated. In the influent, 121 μg L^{-1} MCA and 15 μg L^{-1} DCA were detected. In the activated sludge, an increase in MCA of 275 μg L^{-1} was detected, but there was no increase in DCA. Trichloroacetic acid (TCA) was not detected.

What is so exceptional about this sewage treatment plant that it gives rise to so many chlorinated compounds, especially the 3,4-CPAc? A significant source of unchlorinated compounds to this treatment plant appears to be from a slaughterhouse. The effluent from this slaughterhouse contains phenylacetic acid (PAc) at concentrations up to 60 mg L^{-1}. PAc is found in blood (Davis et al. 1982) and urine (Iwata et al. 1994). Most of the PAc in the influent of the sewage treatment plant originates from the slaughterhouse, as indicated by an increase in PAc in the influent to the treatment plant from 1,030 to 8,500 μg L^{-1} immediately after the slaughterhouse recommenced activities following a 4 day work break in 1998.

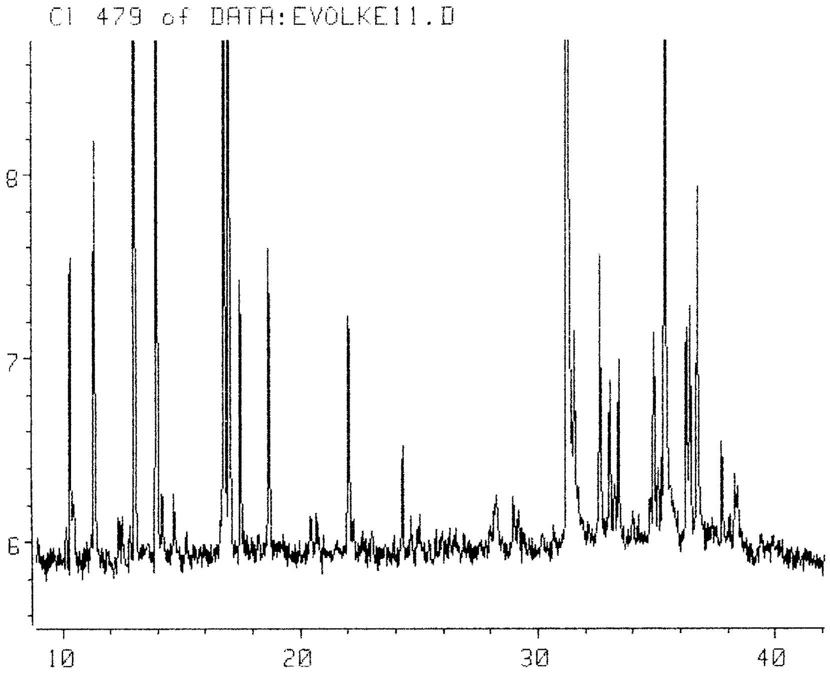


Figure 2. GC/AED chromatogram, Cl-trace.

Artifactual formation of 3,4-CPAc from phenylacetic acid during the analytical procedure can be excluded because the slaughterhouse wastewater possessed both the lowest concentrations of 3,4-CPAc and the highest concentrations of PAc observed, e.g. 40,200 μg L^{-1} PAc and only 33 μg L^{-1} 3,4-CPAc were detected in October 1997. This is in contrast to the activated sludge, where 15,800 μg L^{-1} phenylacetic acid and 3,180 μg L^{-1} 3,4-CPAc were found.

Model experiments

The model chlorination experiments with HOCl and phenylacetic acid revealed that only mono-chlorinated compounds were formed in the reaction with 50 μL NaOCl. Ortho-, para, and α-CPAc (see Figure 5) were formed as the main products. Meta-CPAc was formed only in small amounts. With 250 μL HOCl, dichlorinated isomers were formed, but these compounds were most likely α-aryl-dichloro and not dichloro-aryl substituted. Their mass spectra differed from the spectra obtained for the dichloro-aryl isomers used as standards, with no intensity appearing at m/z = 159, which is typical for dichloro-aryl isomers (see mass spectrum, Figure 3).

Table 1. Concentrations of the identified compounds in the activated sludge (AS) and in the influent (IF) of the sewage treatment plant.

	AS 12/18/96 mg/kg DW	AS 07/02/97 mg/kg DW	AS 07/22/97 mg/kg DW	IF 12/18/96 mg/L	IF 07/02/97 mg/L	IF 07/22/97 mg/L
3-Ald	n.d.	0.05	n.d.	n.d.	n.d.	n.d.
2,3-Ald	14.9	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-Ald	7.21	n.d.	n.d.	n.d.	n.d.	n.d.
3,4-Ald	9.42	0.16	1.04	0.042	n.d.	n.d.
3,5-Ald	8.31	n.d.	n.d.	n.d.	n.d.	n.d.
3,4-Nitril	1.05	n.d.	n.d.	n.d.	n.d.	n.d.
3,4-AcPhenon	0.17	0.05	n.d.	n.d.	n.d.	n.d.
2-CBA	0.12	n.d.	n.d.	n.d.	n.d.	n.d.
3+4-CBA	0.35	0.47	0.21	0.004	0.031	0.003
2,4-CBA	0.87	0.21	0.17	n.d.	0.022	0.003
3,4-CBA	7.79	5.21	3.19	0.011	n.d.	0.001
3,5-CBA	1.92	n.d.	n.d.	n.d.	n.d.	n.d.
3,4-Phenol	n.d.	0.73	n.d.	n.d.	n.d.	n.d.
3-CPAc	25.0	0.83	3.92	0.160	n.d.	n.d.
4-CPAc	3.31	0.21	1.35	0.092	n.d.	n.d.
3,4-CPAc	1090	51.7	175	1.15	0.023	0.006

AS: activated sludge, IF: influent, DW: dryweight, n.d.: not detectable

Ald: chlorobenzaldehyde, Nitril: chlorobenzonitrile, AcPhenon: chloroacetophenone, CBA: chlorobenzoic acid, Phenol: chlorophenol, CPAc: chlorophenylacetic acid

Discussion

The treatment plant investigated in this study is a source of organohalogens, as a net production occurs in it. It appears that this production is biotic, as abiotic model chlorination reactions with HOCl did not lead to the formation of the compounds found in the sewage treatment plant. In addition, there is no other simple aqueous abiotic reaction known to occur that leads to chlorination in the 3,4-position. Further research to determine the microbiological origins of this reaction are needed.

Some of the identified compounds are known to be produced naturally, e.g. 3,4-CPAc can be formed in compost (Nagaoka et al. 1996), and was shown to have an inhibitory effect on seed germination. Chlorinated benzoic acids (CBA) were detected in that study as well. These CBA (3,4- and 3,4,5-CBA) were identical to those found in this work. A trichlorobenzoic acid was also present in several samples of plant material from remote areas (Flodin et al. 1997). Of the other identified organohalogens, dichloroacetic acid (Hoekstra et al. 1995), 2,4- and 3,4-dichlorophenol (Ando et al. 1970; Corgiat et al. 1993) and 2,4-CBA (Flodin et al. 1997; Niedan and Schöler 1997) are known to be formed naturally. 3,4-CPAc has been identified in fungi (Niedan 1998). As we applied the oxidative degradation method, which is able to degrade macromolecules into phenolic monomers (Dahlmann et

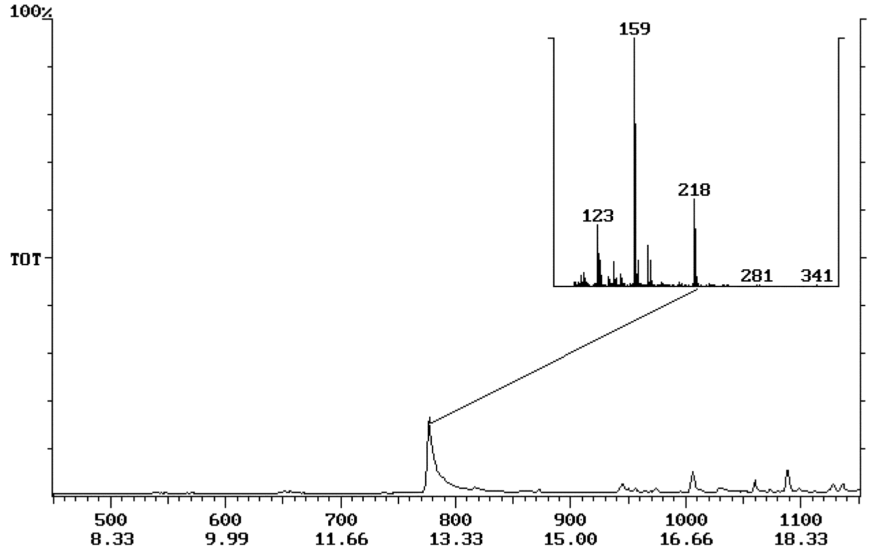


Figure 3. GC/MS chromatogram of an activated sludge sample (total ion current) with mass spectra of 3,4-CPAc; The molpeak of the methylester is 218, m/z = 159 corresponds to $m^+ - COOCH_3$.

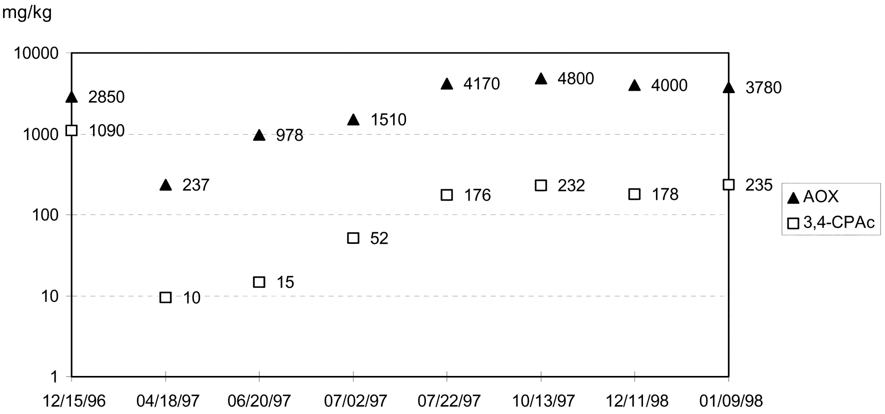
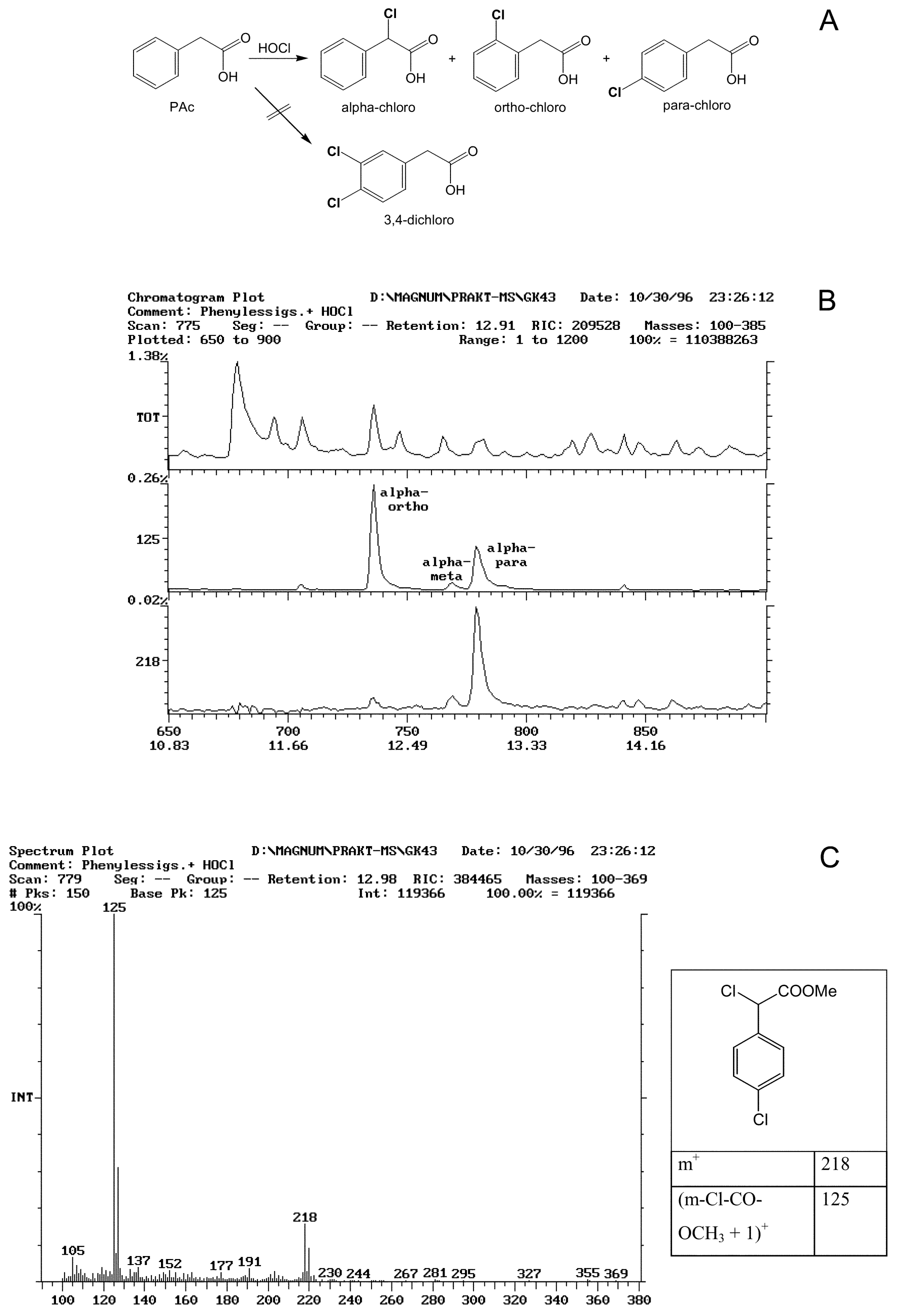


Figure 4. AOX and 3,4-CPAc of the activated sludge in mg kg^{-1} dryweight.

al. 1993), similar chlorinated structures known to be of natural origin, e.g. from humic substances (Flodin et al. 1997), were found in the sludge.

That the AOX-balance shows a net increase in the sewage treatment plant suggests that the identified compounds are not metabolites of other chlorinated compounds emitted to the sewage treatment plant, but they rather are generated de novo. In particular a balance of the 3,4-CPAc showed that this compound was formed in the treatment plant. While the average 3,4-CPAc concentration of the influent to the plant (0,0145 mg L^{-1} from the two samples from July '97) indicated that 55 g d^{-1} 3,4-CPAc were transported to the plant (in the 3,800 m^3 wastewater), 453



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Chromatogram Plot          D:\MAGNUM\PRAKT-MS\GK43   Date: 10/30/96  23:26:12
Comment: Phenylessigs.+ HOCl
Scan: 775    Seg: --  Group: -- Retention: 12.91  RIC: 209528   Masses: 100-385
Plotted: 650 to 900                       Range: 1 to 1200     100% = 110388263
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Spectrum Plot              D:\MAGNUM\PRAKT-MS\GK43   Date: 10/30/96  23:26:12
Comment: Phenylessigs.+ HOCl
Scan: 779    Seg: --  Group: -- Retention: 12.98  RIC: 384465   Masses: 100-369
# Pks: 150    Base Pk: 125                Int: 119366     100.00% = 119366
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m^+	218
$(m\text{-}Cl\text{-}CO\text{-}OCH_3 + 1)^+$	125

Figure 5. a) reaction scheme of the chlorination of Phenylacetic acid (PAc) with HOCl. b) Chromatogram of the reaction of PAc with 250 μL HOCl with total ion current, m/z = 125 ($m^+ - COOCH_3$) and m/z = 218 (m^+). c) mass spectra of α-p-dichlorophenylacetic acid methylester.

g d^{-1} were emitted daily (with the produced 4 t of dried sludge) (see Table 1). In most cases (except for January '98) an increase in 3,4-CPAc was observed if AOX values increased (Figure 4).

It appears that the phenylacetic acid emitted from the slaughterhouse is largely responsible for this production of 3,4-CPAc, as it is the probable educt for the 3,4-CPAc in the biosynthesis. Krause (2000) also detected 3,4-CPAc in sewage treatment plants in Heidelberg. In this study, 3,4-CPAc was detected at low levels (0.6 μg L^{-1}) in activated sludge of treatment plants that did not process slaughterhouse effluent, whereas levels up to 800 μg L^{-1} 3,4-CPAc were observed in the activated sludge of plants receiving slaughterhouse effluent.

That 3,4-CPAc has an inhibitory effect on germination of some seeds is of interest if sewage sludge with high concentrations of this compound are to be used in agriculture. Due to the large numbers of slaughterhouses, whose wastewater is processed by sewage treatment plants, a significant source of organohalogens to the environment could exist which has not been considered before.

Acknowledgements

The authors would like to thank Dr Chris Swartz and Dipl. Min., MES Isabelle Fahimi for the helpful correction of the manuscript.

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