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The Determination of Pentachlorophenol and Tetrachlorophenols in Wadden Sediment and Clams (*Mya arenaria*) Using Triethylsulfonium Hydroxide for Extraction and Pyrolytic Ethylation†

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A method to determine the concentration of pentachlorophenol and tetrachlorophenols in wadden sediments and clams is described. This method involves the extraction of lyophilized specimens with toluene under acidic conditions and the back extraction of the chlorophenols into a methanol/water solution of triethylsulfonium hydroxide. Upon injection of the methanol/water phase into the gaschromatograph a pyrolytic ethylation is performed and the ethylethers of pentachlorophenol and tetrachlorophenols formed thereby are separated in quartz capillary columns and detected by an electron capture detector. Using tribromophenol as internal standard the recovery rates for the chlorophenols were within the range of 76.7 and 98.8%. The method described does not require any evaporation or chromatographic clean-up steps. The detection limit was found to be 2 nmol/kg (~500 ng/kg) for sediment and 0.1 μ mol/kg (~25 μ g/kg) for clams. Its accuracy was verified by gaschromatography-mass spectrometry experiments.

KEY WORDS: Pentachlorophenol, tetrachlorophenol, triethylsulfonium hydroxide, pyrolytic ethylation.

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INTRODUCTION

Pentachlorophenol is a priority pollutant according to a high annual production rate of about 100,000 tons¹ and its persistence, which has resulted in contamination of water,²⁻⁴ sediment^{5,6} and aquatic organisms.^{5,7-10} It is used extensively as fungicide, bactericide, insecticide, herbicide and wood preservative. The total amount of chlorophenols produced is even higher, exceeding 200,000 tons per year.¹¹

Apart from the global pollution caused by these substances, local phenomena give reason for growing concern. Such case study was the starting point for the investigation presented here.

In the years from 1965-1978 waste water of a paper mill containing pentachlorophenol (PCP) was drained off into the mud flat of the "Jadebusen", a bight of the German shore. During this time about 26 tons of PCP were discharged. In November 1978 the use of PCP was stopped.

The goal of our investigation was to determine the concentration of PCP in wadden sediment and clams and to characterize and quantify its metabolites. These were supposed to be tetrachlorophenols (TeCPs), especially 2.3.4.5 TeCP and 2.3.5.6 TeCP that are known to be formed under anaerobic conditions.^{12,13} 2.3.4.6 TeCP may be a constituent of the waste water, since it is known to be a technical impurity in PCP.¹⁴

To identify and quantify PCP in ecological matrices like sediments and organisms various methods have been described. Most of them rely on gas liquid chromatography (GLC) with electron capture detection (ECD). For the GLC separation derivatization with acetanhydride,^{2,3,15} trimethylchlorosilane,¹⁶ diazomethane^{9,10,16-18} and diazoethane^{5,19} has been reported. For clean up acid alumina,^{9,17,18} silica gel,¹⁸ Florisil,^{10,19} anion exchange resins⁵ or even steam distillation⁶ has been used. However, these methods are rather time consuming or use hazardous reagents like diazo compounds. Taking our studies with pyrolytic alkylation reagents as a basis,²⁰ we would like to report here accuracy, precision, detection limits and recovery rates for a very simple method to analyse PCP and TeCPs in sediments and clams with Triethylsulfonium Hydroxide (TESH) as an extraction and derivatization reagent.

MATERIAL AND METHODS

Gas liquid chromatography

Apparatus A Varian (3700) and a Siemens (L 402) gaschromatograph, both equipped with a capillary injection block and an ECD (⁶³Ni) were used for the analytical work. Capillary quartz columns (Chrompack) of

25 m length and an I.D. of 0.2 mm coated with CP 51tm and OV 101 were applied for the separation.

Conditions Injection Port temp.: 250°C, detector temp.: 300°C, carrier gas: hydrogen with a linear flow of 50 cm/sec, injection mode: split (1:50) or splitless, make up gas: nitrogen (Varian 20 ml/min, Siemens: 40 ml/min).

Column temperatures: OV 101: 3 min at 120°C, 5°C/min to 200°C,
CP 51tm: 5 min at 140°C, 5°C/min to 190°C.

Calculations The PCP- and TeCP-concentrations in the samples were calculated using the peak height ratio between the chlorophenoethylethers and the 2.4.6 tribromophenoethylether. 2.4.6 tribromophenol (TBP) served as internal standard.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) experiments were performed with a Varian 3700 GC coupled directly to a Varian 44S quadropole mass spectrometer equipped with a Varian SS 200 data system. GC conditions were as described above using helium instead of hydrogen as a carrier gas with a linear flow of 20 cm/sec. MS conditions were: Source 70 eV, emission current: 0.5 μ A, ion source temp.: 200°C. Measurements were performed in the total ion current (TIC) mode as well as in the multiple ion selection (MIS) mode.

Reagents

All reagents were analytical grade (E. Merck). Toluene was further purified by shaking it twice with one tenth the volume of 1 mol/l sodiumhydroxide.

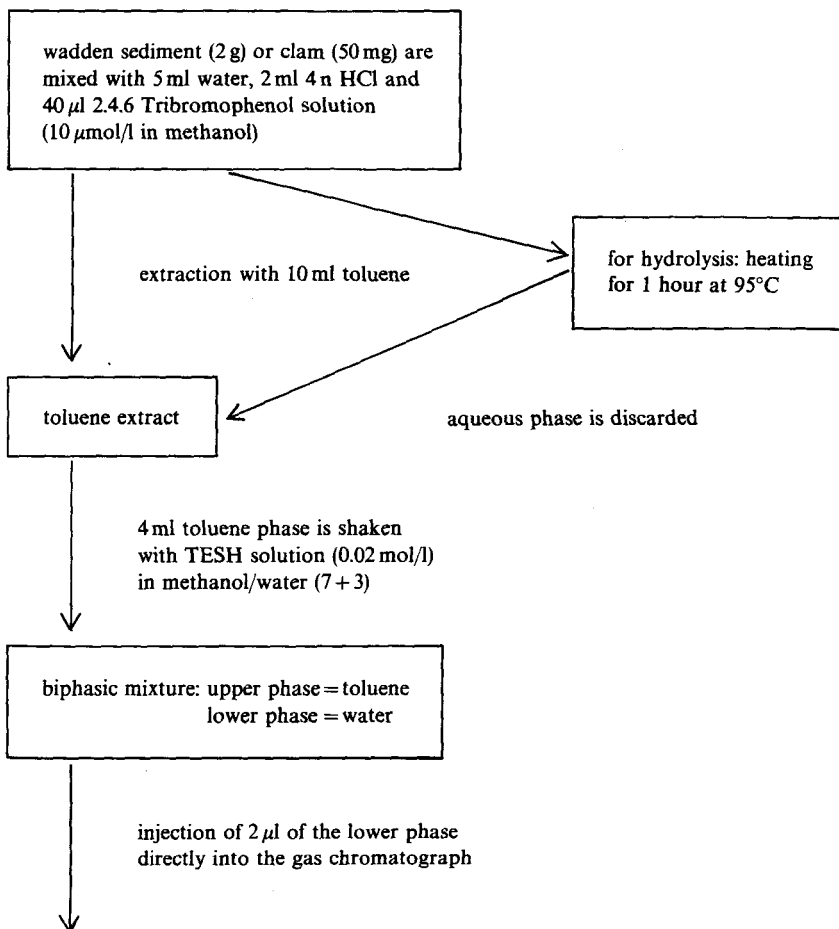
Preparation of TESH Triethylsulfoniumiodide (Alfa/Ventron) was dissolved in methanol/water (7+3) to form a 0.02 mol/l solution. This was stirred with an equimolar amount of silveroxide (E. Merck) for 1 hour at room temperature. The solution of TESH was decanted off from the silveriodide precipitate and stored at 4°C. Under these conditions it is stable at least for 6 months.

Preparation and extraction of the samples

Wadden sediment and clams (*Mya arenaria*) were lyophilized. After homogenizing the wadden sediment in a mortar the samples were

subjected to sieving, yielding in a ≤ 1 mm (whole fraction) and a $\leq 63 \mu\text{m}$ fraction (silt). Particles larger than 1 mm were discarded. Clams (without shells) were homogenized in a ball mill.

SCHEME 1
Extraction of PCP and TeCPs from wadden sediment and clams.



Samples of the wadden sediment and of the clams were extracted according to Scheme 1. It should be noted that some samples were hydrolyzed by heating them at 95°C under strong acidic conditions for one hour.

RESULTS AND DISCUSSION

Separation of PCP and TeCPs

The GLC separation and quantification of traces of TeCPs and PCP without derivatization is not recommended because these compounds are rather strong acids (pK_a of PCP = 4.74; pK_a of TeCPs = 5.03–5.64)²¹ and may be adsorbed irreproducibly during the chromatographic separation. To derivatize the chlorophenols we used pyrolytic ethylation with TESH, a proposal for the reaction mechanism is given in Figure 1. Choosing TBP as an internal standard, the retention times of pentachlorophenoylether, tetrachlorophenoylethers and tribromophenoylether are identical to the retention times of PCP, TeCPs and TBP after pyrolytic ethylation (see Figure 2). However, 2,3,4,6- and 2,3,5,6 TeCP could not be separated, neither with the OV 101 coated quartz capillary column nor with the CP 51tm column. Recovery experiments were therefore performed with 2,3,5,6 TeCP only.

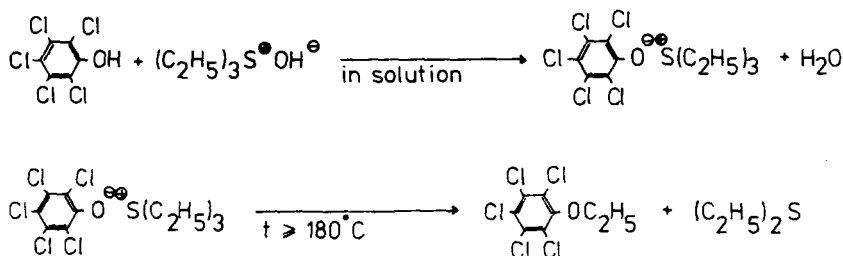


FIGURE 1 Reaction of PCP with TESH.

TESH as an extraction and derivatization reagent

To separate the chlorophenols from organic and anorganic components the sediment or clam samples, suspended in water, were extracted with an organic solvent under acidic conditions. This solvent may be hexane-isopropanol^{6,15} or benzene.^{10,17} Like Stark¹⁶ we used toluene, because it is less toxic than benzene and the solubility of TeCPs and PCP is better in toluene than hexane.

To separate the acidic components, like PCP and TeCPs, from the neutral compounds in the toluene phase a back extraction into an alkaline solution is advisable. We used methanol/water (7+3) containing 0.02 mol/l TESH (with a pK_b of 12.0²⁰) to extract the acidic compounds into a

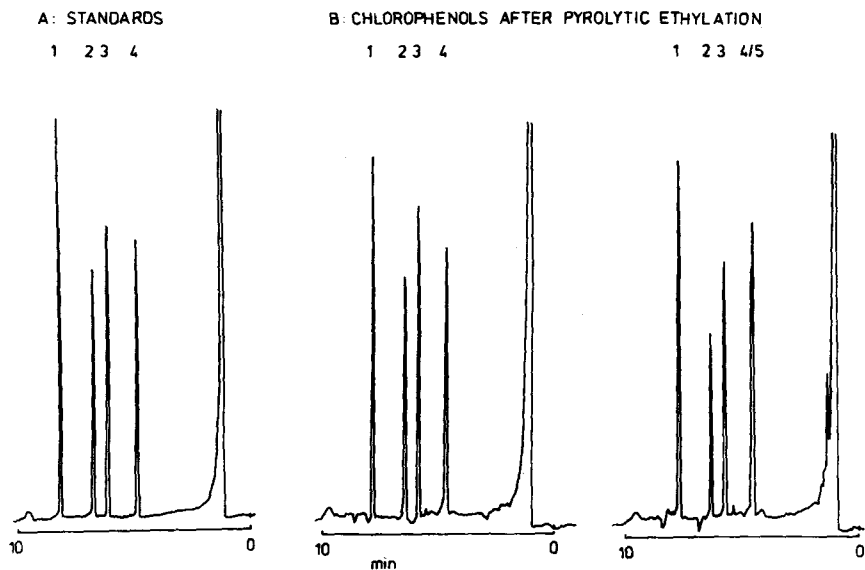


FIGURE 2 Chromatograms of the ECD-GLC analysis of the ethylethers of PCP (1), 2,3,4,5 TeCP (2), TBP (3), 2,3,5,6 TeCP (4) and 2,3,4,6 TeCP (5) respectively.

TABLE I

Yields for the extraction/ethylation of 2,3,5,6 TeCP to its ethylether by TESH.

Given are the peak height ratio of 2,3,5,6 TeCP to TBP for the extraction and ethylation from toluene into methanol/water (7+3, containing TESH, 0.02 mol/l) compared to the peak height ratio of 2,3,5,6 tetrachlorophenoethylether to tribromophenoethylether. Concentrations of TBP or tribromophenoethylether were: 40 nmol/l (see also figure 3, $n=6$).

Concentration of TeCP	Yield (%)
4 nmol/l	88.5 ± 4.6
12 nmol/l	86.4 ± 4.3
20 nmol/l	88.5 ± 4.6
30 nmol/l	88.8 ± 4.6
40 nmol/l	86.3 ± 4.5
60 nmol/l	90.1 ± 6.2
80 nmol/l	88.4 ± 4.6

solvent not mixable with toluene (see Figure 3). As demonstrated in Table 1 for 2,3,5,6 TeCP there is a constant ratio in the yield for the extraction and ethylation of TeCPs and PCP if TBP is used as an internal standard.

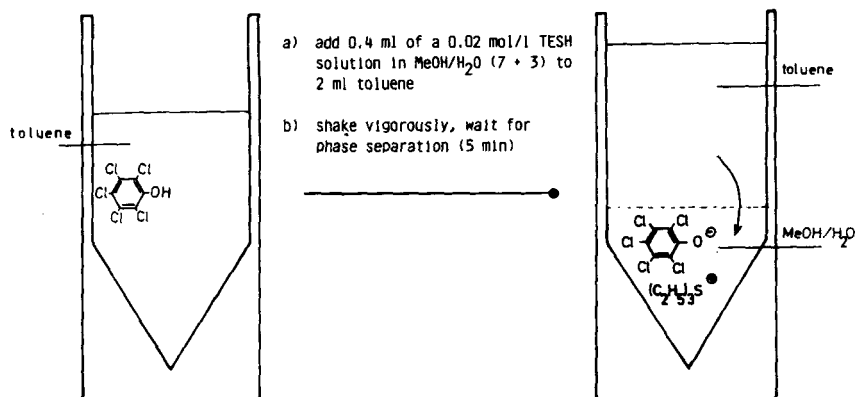


FIGURE 3 Extraction of chlorophenols (e.g. PCP) from toluene into methanol/water with TESH.

Recovery experiments

PCP is known to be volatile upon steam distillation.²² By lyophilizing and reconstituting water samples of pH=8 spiked with 40 nmol/l PCP and TeCPs we found that there was no reduction of the chlorophenol concentration caused by the freeze drying process. To evaluate the recovery rates, samples were extracted according to Scheme 1 and analyzed by GLC with and without the addition of 40 nmol/kg (wadden sediment) or 2.5 μ mol/kg (clam). The results are listed in Table 2. Results

TABLE II

Recovery rates (%) for chlorophenols from wadden sediment and clam.

40 nmol/kg of the chlorophenols were added to 2 g of wadden sediment, 2.5 μ mol/kg to 50 mg of clam respectively.

	PCP	2,3,4,5- TeCP	2,3,5,6- TeCP	
Sediment (whole fract.)	82.5 \pm 4.1	86.7 \pm 4.1	82.3 \pm 3.4	n=6
Sediment (silt fract.)	81.2 \pm 7.2	79.4 \pm 1.9	76.7 \pm 3.5	n=4
Clam	79.5 \pm 4.5	87.0 \pm 6.8	84.4 \pm 3.4	n=6

Listed are the mean value and (\pm) the 95% confidence interval.

for samples, that were hydrolyzed are compiled in Table 3. Recovery rates for TeCPs and PCP were between 76.7% and 87.0% for specimens not hydrolyzed and between 84.5% and 98.8% for hydrolyzed samples.

TABLE III

Recovery rates (%) for chlorophenols from wadden sediment and clams (hydrolyzed specimens).

40 nmol/kg of the chlorophenols were added to 2 g of wadden sediment, 2.5 μ mol/kg to 50 mg of clam respectively.

	PCP	2.3.4.5- TeCP	2.3.5.6- TeCP	
Sediment (whole fract.)	92.8 \pm 5.1	84.5 \pm 3.6	96.4 \pm 3.4	n = 22
Sediment (silt fract.)	93.2 \pm 4.4	87.6 \pm 3.5	98.8 \pm 4.4	n = 13
Clam	97.6 \pm 3.6	87.6 \pm 3.7	94.6 \pm 3.4	n = 16

Listed are the mean value and (\pm) the 95% confidence interval.

Precision, accuracy and detection limits

The precision for the method described may be obtained from Tables 2 and 3 regarding the 95% confidence interval (CI). The CI for a mean value of n measurements is connected to the standard deviation s by: $CI = s \cdot t / \sqrt{n}$, with t being the value of the two-sided t -statistics. Thus, standard deviations for a 40 nmol/kg concentration of PCP and TeCPs in wadden sediment were 2 nmol/kg. The standard deviation for a 2.5 μ mol/kg concentration in clams was 0.12 μ mol/kg. Therefore precision between runs had a coefficient of variation of about 5%.

The detection limit, calculated as a peak greater than 3 fold the base noise, was estimated as 2 nmol/kg (\sim 500 ng/kg) for wadden sediment and 0.1 μ mol/kg (\sim 25 μ g/kg) for clams. With some modifications in the extraction procedure or with an evaporation step it will be possible to increase the sensitivity at least by a factor of 10.

The identification of the PCP- and TeCP-ethylether peaks was possible regarding the identical retention times obtained with two separation phases of different polarity (OV 101 and CP 51tm). Definite evidence was received from GC-MS experiments using the MIS mode. The highly chlorinated phenols have a characteristic ratio of isotopes, because of the natural ratio of ³⁵Cl and ³⁷Cl (3:1). The mass spectrum of the ethylether of PCP (see Figure 4) shows molecule ions of 292/294/296 and 298 with the even more intense fragment ions 264/266/268 and 270 produced

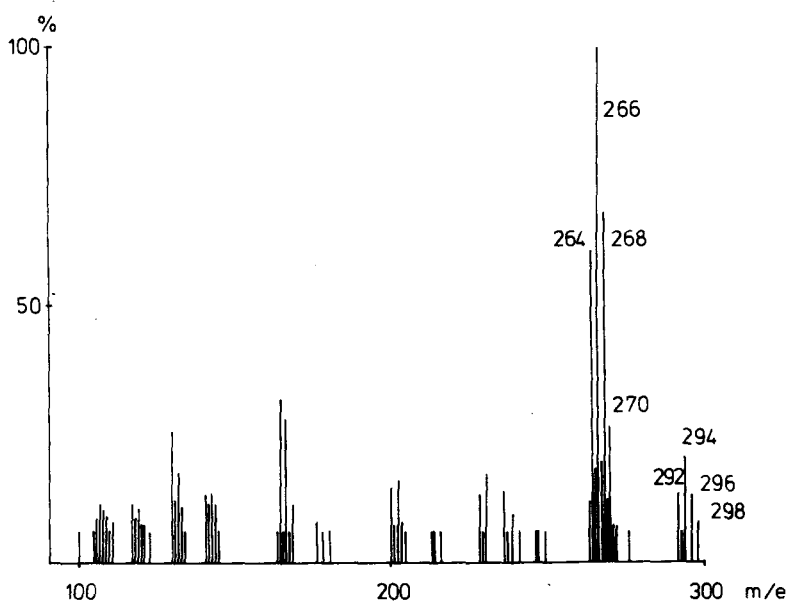


FIGURE 4 Mass spectrum of pentachlorophenoethylether.

through the split off of ethylene. The corresponding highly intense fragment ions of the ethylethers of the TeCPs are 230/232 and 234. As demonstrated in Figure 5, the ions of the reference samples and the ethylated extracts from sediment have identical retention times.

GLC analyses of extracts from wadden sediment and clams

Examples for the GLC separation of TeCPs and PCP from an extract of wadden sediment and of a clam are given in Figure 6 and Figure 7 respectively. Using the working up procedure discussed above, neutral substances that could interfere with the gas chromatographic separation of TeCPs and PCP are excluded. Acidic compounds, other than chlorophenols, being detectable by an ECD, were not mentioned.

PCP concentrations near the end of the sewage tube were between 20 and 60 nmol/kg for wadden sediment and between 1 and 12 μ mol/kg for clams (values on dry weight basis). Concentrations for 2,3,4,5 TeCP were between 100 and 140 nmol/kg in wadden sediments and 2 and 200 μ mol/kg for clams, whereas the concentrations for 2,3,5,6/2,3,4,6 TeCP did not exceed the detection limit.

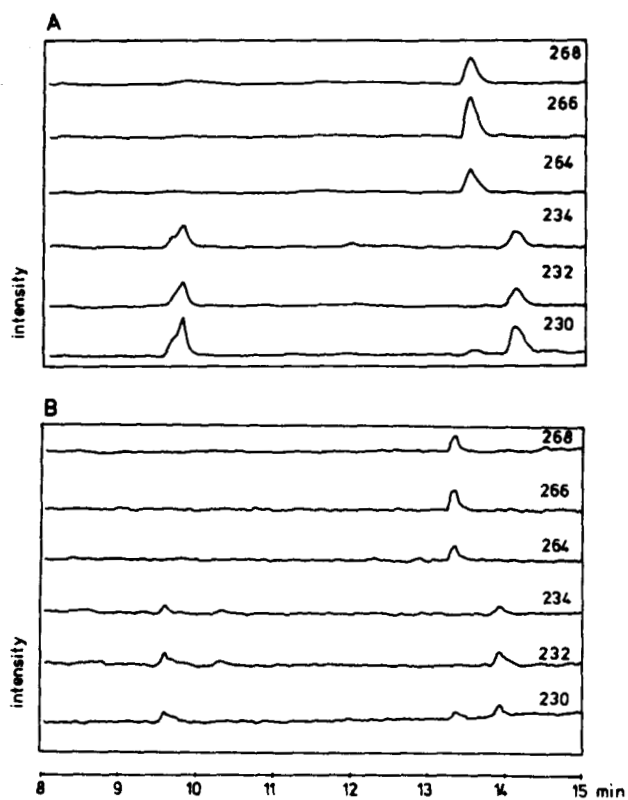


FIGURE 5 Chromatograms obtained in the MIS mode for pentachlorophenoethylether (m/e: 266/264/262) and tetrachlorophenoethylethers (m/e: 234/232/230). A.: Standards, B.; Extract from wadden sediment after pyrolytic ethylation using TESH.

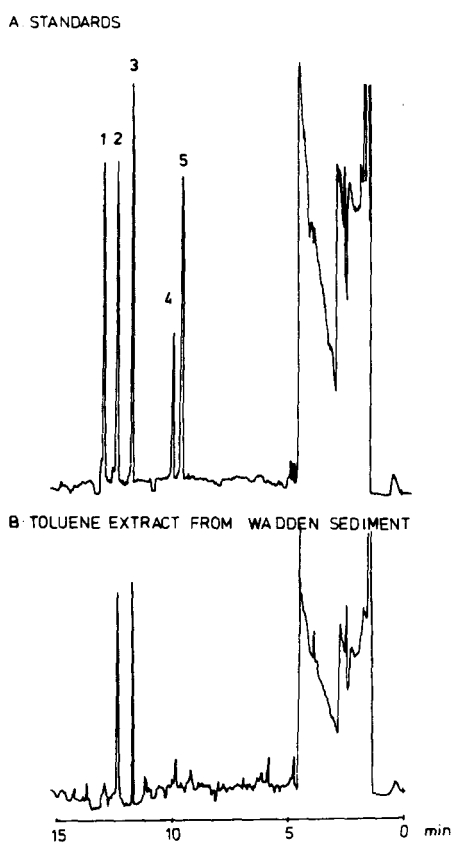


FIGURE 6 Chromatogram of the ECD-GLC analysis of a toluene extract from wadden sediment (splitless injection) compared to reference substances: 2,3,5,6 TeCP (1), PCP (2), TBP (3), 3,4,5 trichlorophenol (4) and 2,3,4,5 TeCP (5).

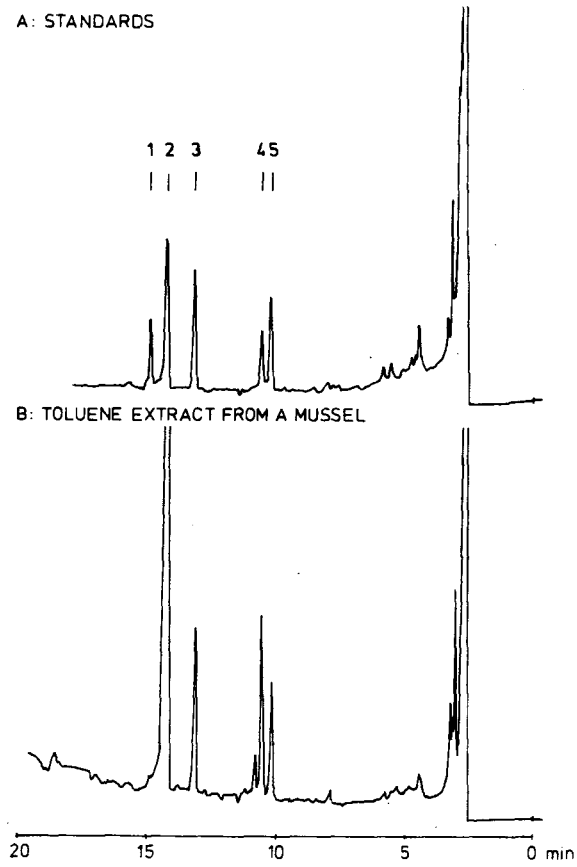


FIGURE 7 Chromatogram of the ECD-GLC analysis of a toluene extract of a clam (hydrolyzed specimen) compared to reference substances: 2.3.5.6 TeCP (1), PCP (2), TBP (3), 3.4.5 trichlorophenol (4) and 2.3.4.5 TeCP (5).

A detailed discussion of TeCP and PCP concentrations for specimens obtained near the end of the sewage tube and from reference places of the "Jadebusen" also regarding the effect of hydrolysis will be published elsewhere.²³

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