Toxic Effects of Pollutants on the Mineralization of Chloroform in River Sediments

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The influence of pollutants on the formation of "CO₂ from 3 μg/liter labeled chloroform was studied in anaerobic Dutch river sediments. All incubations were performed under anaerobic conditions. Addition of toxicants to sediment microcosms showed logistic dose-effect curves. The concentration giving 10% inhibition of the chloroform mineralization rate (IC₁₀) was derived from these dose-effect curves. The IC₁₀ values of added cadmium, chloropyrifos, benzene, mercury, or 1,2-dichloroethane were 1300, 1300, 140, 90, and 0.07 mg/kg dry sediment, respectively. Mud samples taken at different dates from the same site indicated a significantly different sensitivity to added pentachlorophenol and zinc. The IC₁₀ of added pentachlorophenol was 150 mg/kg in one and 15 mg/kg in another sample. Chloroform-mineralizing bacteria are very sensitive to addition of zinc. The IC₅₀ of added zinc was 700 mg/kg for one sample and 11 mg/kg for another sample of the sediment which contained a background concentration of 800 mg Zn/kg. Therefore, a partial inhibition of the mineralization of chloroform by the high concentrations of zinc present in Dutch river sediments cannot be excluded. The high concentration of zinc might cause persistence of otherwise biodegradable pollutants in Dutch sediments. © 1994 Academic Press, Inc.

INTRODUCTION

The pollution of river sediments from the Rhine and the Meuse has become a major problem in Holland. The total load of suspended matter of the river Rhine varies an annual average of between 50 and 150 kg/sec (Van der Weijden and Middelburg, 1989). Numerous pollutants are sorbed to particles and are sedimented in the Biesbosch, the Ketelmeer, and the harbors of Rotterdam. A large part of the 20*10⁶ m³ of sediment that is dredged each year from the harbor area of Rotterdam must be treated as chemical waste. Storage or treatment of this sediment is a major problem. The distinction between polluted and unpolluted sediments is based on sediment quality standards. These quality standards should be based on the risk of a pollutant for man or the environment. A pollutant concentration which decreases the self-purifying capacity of sediments imposes a risk for the river ecosystem.

In anaerobic river sediments a reductive dechlorination of many chlorinated organic pollutants occurs (Kohring et al., 1989; Struijs and Rogers, 1989; Peijnenburg et al., 1991). Chloroform is a common pollutant present in concentrations of 1–50 μg/liter in the Rhine (RIZA, 1986). It is volatile and does not sorb strongly to sediment (Van Beelen and Van Keulen, 1990). In groundwaters and sediments the volatilization of chloroform is very slow because of limited exchange with the atmosphere. In methanogenic sediments chloroform can be mineralized at low concentrations to carbon dioxide, methane, and a low amount of dichloromethane (Bouwer and McCarty, 1983; Van Beelen and Van Vlaardingen, 1993; Galli and McCarty, 1989). Chloroform is not only a substrate for some species of bacteria but also a toxicant for many other
bacteria. Chloroform is able to inhibit methane production at 500 µg/liter anaerobic digester sludge (Yang and Speece, 1986). The EC\textsubscript{10} of chloroform on methane production is 5.5 mg/kg sediment (Van Vlaardingen and Van Beelen, 1992). The degradation of chloroform is, therefore, an important process in anaerobic sediments.

This paper describes the effect of various pollutants on the mineralization of chloroform in anaerobic river sediments. This knowledge can be used to set sediment quality standards which do not allow inhibition of the microbial activities which are vital for the self-purifying capacity of the river ecosystem.

MATERIALS AND METHODS

Chemicals

Chloroform, \(^{14}\)CHCl\(_3\) (Amersham), had a chemical purity of 97% and specific activity of 2.44 MBq/mg. Chloropyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate) (Riedel de Haen, 89\%) was dissolved in dimethyl sulfoxide (DMSO). Benzene (Merck, 99.7\%) was added pure with a micropipet. HgCl\(_2\) (Merck, 99.5\%) was dissolved in 1 N HCl. CdCl\(_2\)\cdot\text{H}_2\text{O} (Merck, 98\%) was dissolved in double-distilled water. ZnCl\(_2\) (Merck, 98\%) was dissolved in 0.1 N HCl and pentachlorophenol (PCP) (Fluka, 99\%) was dissolved in 0.5 N NaOH.

Sampling Site and Methods

The methanogenic mud samples were obtained from a depth of 10–40 cm below the water sediment interface in a little harbor in the Rhine delta near Gorinchem as described previously (Van Beelen and Van Keulen, 1990). Fresh mud was mixed with an equal weight of anaerobic double-distilled water and 20 ml of homogenized suspension was pipetted into incubation bottles in an anaerobic glove box under nitrogen. For the toxicity experiments, portions of stock solutions of a toxicant were pipetted in bottles 2 hr before addition of labeled chloroform. Addition of toxicants did not change the pH more than 0.1 unit. The final concentrations of \(^{14}\)CHCl\(_3\) in the incubation bottles were 2.7 to 3.4 µg/liter and 133 to 167 Bq per bottle with 20 ml suspension, respectively.

Measuring the Mineralization

Bottles without toxicant were incubated at 20°C in a shaker to determine the half-life of chloroform. The bottles with toxicant were incubated under the same conditions. For each time or toxicant concentration two bottles were analyzed. The mineralization was terminated by freezing incubation bottles upside down at −20°C. For the extraction of carbon dioxide 5 ml of a 1/10 mixture of silicon antifoam and 4 N H\(_2\)SO\(_4\) was added and the bottles were heated to 80°C. Nitrogen gas was used to flush the formed \(^{14}\)CO\(_2\) out of the incubation bottles. The gas was led through two gas washing vials containing 3 ml of hexane + 7 ml of instagel + 1 drop of 1 N HCl, and subsequently through two gas washing vials containing an ethanamine-based CO\(_2\) absorbent. Liquid scintillation counting was used to measure radioactivity. The radioactivity in the hexane was counted as volatile chloroform and in the ethanamine as carbon dioxide. The verification of the method and the experimental procedure were described in more detail previously (Van Beelen and Van Keulen, 1990; Van Beelen and Van Vlaardingen, 1993). The half-life of the chloroform mineralization was obtained from the measured
labeled chloroform and the formed carbon dioxide as described previously (Van Beelen and Van Vlaardingen, 1993).

Dose-Effect Relations

The toxic effect of a pollutant on organisms can be quantified by experiments in which identical groups of organisms are exposed to increasing concentrations of the pollutant. When the toxicant concentration (the dose) is plotted on the X axis and the effect on the Y axis, a dose–effect curve is obtained. Similarly, identical bottles with sediment can be exposed to increasing concentrations of a pollutant and the mineralization of a substrate such as chloroform can be monitored by measuring the substrate concentration and the carbon dioxide formed. The effect of toxicants on the mineralization of a substrate can be described by the following logistic model (Van Beelen et al., 1991; Haanstra et al., 1985):

\[
\%P(c) = \%b / (1 + \exp\{\text{slope} \times \left[\log(\%c/\text{EC}_{50})\right]\})
\]

\(c\) = concentration of the toxicant in mg/kg
\(\%P(c)\) = percentage carbon dioxide produced during the incubation time at a toxicant concentration \(c\).
\(\%b\) = the percentage carbon dioxide produced without addition of a toxicant.
\(\text{EC}_{50}\) = that concentration of the toxicant which causes a 50% reduction of the carbon dioxide produced at a certain incubation time.
\text{slope} = a constant which determines the steepness of the dose–response curve.

When the \(\text{EC}_{50}\) and the \(\text{EC}_{10}\) are known, the slope can be determined as follows: at the toxicant concentration \(\text{EC}_{10}\) the percentage carbon dioxide formed after a certain incubation time is only 90% of the control. Inserting \(c = \text{EC}_{10}\) and \(\%P(c)/\%b = 90/100\) in Eq. (1) gives:

\[
\text{slope} = \ln(9)/\log(\text{EC}_{50}/\text{EC}_{10}).
\]

The disappearance of the substrate can be described by a similar dose–response curve.

\[
\%S(c) = 100 - (100 \times \%P(c)/\%\text{max})
\]

\(\%S(c)\) = percentage chloroform degraded during the incubation time at a toxicant concentration \(c\).
\(\%\text{max}\) = the maximal percentage of carbon dioxide formed from 100% mineralization of the substrate, calculated from the control measurement without toxicant.

For dose–effect data the nonlinear least-squares method was used on \(\%P(c)\) and \(\%S(c)\) simultaneously (Van Beelen et al., 1991) since both curves are coupled. Standard deviations of \(\text{EC}_{50}\), \(\text{EC}_{10}\) and half-lives were determined by splitting the duplicate data set in two and calculating either the \(\text{EC}_{50}\) and \(\text{EC}_{10}\) or the half-lives from a single data set. The standard deviation of the resulting duplicate values was calculated using the unbiased \((n - 1)\) method.

The Effect of the Incubation Time on the Dose-Effect Relation

The measured \(\text{EC}_{50}\) and \(\text{EC}_{10}\) toxicant concentrations increase at prolonged incubation times since the un intoxicated part of the microflora continues to mineralize the substrate. A better way to express the effect of a toxicant on a mineralization
process is to measure the effect of a toxicant on the rate of the mineralization process. This is very laborious since the amount of substrate left should be measured at many incubation times and toxicant concentrations. This approach was reported for the degradation of 2,4-dichlorophenoxyacetic acid methyl ester in water (Said and Lewis, 1991). The IC$_{10}$ and IC$_{50}$ are defined as the toxicant concentrations which decrease the mineralization rate by 10 or 50%, respectively (Van Beelen et al., 1991). The definition of the IC$_{50}$ is identical to the half-life doubling (Said and Lewis, 1991). At very short incubation times the EC$_{50}$ becomes similar to the IC$_{50}$ (Van Beelen et al., 1991) and, therefore, it would be better to use very short incubation times. In practice, however, only a small percentage of the substrate is mineralized at very short incubation times. This small percentage can be measured accurately only when $^{14}$C-labeled substrate is used with a very high specific activity and radiochemical purity. At longer incubation times more substrate is mineralized but the measured EC$_{50}$ and EC$_{10}$ will increase. When the uninhibited half-life of the mineralization is known, the IC$_{50}$ and IC$_{10}$ values can be calculated from the EC$_{50}$ and EC$_{10}$ values using the formulas derived previously (Van Beelen et al., 1991). This allows the IC$_{50}$ and IC$_{10}$ to be derived from experiments with longer incubation times. The standard deviation of the IC$_{50}$ and IC$_{10}$ values are calculated using the duplicate EC$_{50}$, EC$_{10}$, and the half-life values. The combination of two logistic curves with two first-order curves results in four possible sets of IC$_{10}$ and IC$_{50}$ values. The standard deviation of the IC$_{10}$ or IC$_{50}$ was calculated from these four values using the unbiased ($n-1$) method.

RESULTS AND DISCUSSION

*The Effect of Pentachlorophenol on the Mineralization of Chloroform*

Figure 1 indicates the effect of pentachlorophenol on the mineralization of chloroform in the sediment sample G-mud-C. The data of the dose–effect relation could be fit by a logistic curve. Note that the dose–effect curve shows a very gradual increase of the effect. There is a large difference between the EC$_{50}$ of 350 mg pentachlorophenol/kg sediment and the EC$_{10}$ of 43 mg/kg. The chloroform mineralization in this sediment sample showed a half-life of 4.5 days and the incubation time of the bottles with pentachlorophenol was 13 days. The time-dependent EC$_{50}$ and EC$_{10}$ concentrations

![Graph](image.png)

**FIG. 1.** The effect of pentachlorophenol on the mineralization of chloroform. EC$_{50}$ = 350 mg/kg; EC$_{10}$ = 43 mg/kg.
were used to calculate the IC\textsubscript{50} and IC\textsubscript{10} which represent the toxicant concentrations which give a 50 or 10% decrease of the first-order mineralization rate. Table 1, line 7, indicates the IC\textsubscript{50} and IC\textsubscript{10} that were calculated from these data. The table summarizes the results of eight dose–effect relations with four samples from the methanogenic Gorinchem sediment taken in 1987, 1989, and 1990. The effect of pentachlorophenol differed significantly between sample G-mud-B and G-mud-C shown in Table 1 (Student's \textit{t} test on separate IC\textsubscript{50} values). Sediment analyses indicated relatively small differences between samples which contained 3–5% organic carbon, 23–28% clay, and 9–11% chalk at pH 7.4–8.1 (Van Beelen and Van Vlaardingen, 1993). The reproducibility of the toxic effects between the duplicate bottles containing the same homogenized sediment sample is much better than the reproducibility between different samples taken at different times from the same location. This in accordance with our previous study on the effect of pentachlorophenol on the mineralization of acetate in soils (Van Beelen and Fleuren-Kemilä, 1993). When pentachlorophenol was added a week before the chloroform, the toxic effect remained unchanged (see Table 1). This indicates that biodegradation or slow sorption did not decrease the bioavailability of pentachlorophenol in this period. For pentachlorophenol the highest reported concentration in Dutch river sediment was 34 \textmu g/kg (Wegman and Van den Broek, 1983). Hence, no direct effect of pentachlorophenol is expected in these sediments.

*The Effect of Chloropyrifos*

Figure 2 demonstrates the effect of chloropyrifos on the chloroform mineralization. Only at very high doses is an effect noted. When the toxicant was added a week before addition of labeled chloroform, no toxic effect of 4000 mg chloropyrifos/kg sediment was noted. Sorption or biodegradation during preincubation might have prevented

<table>
<thead>
<tr>
<th>Compound and sediment</th>
<th>EC\textsubscript{50} (mg/kg)</th>
<th>EC\textsubscript{10} (mg/kg)</th>
<th>Inc (days)</th>
<th>IC\textsubscript{50} (mg/kg)</th>
<th>SD</th>
<th>IC\textsubscript{10} (mg/kg)</th>
<th>SD</th>
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<tr>
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<td>7</td>
<td>6</td>
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<tr>
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<td>12</td>
<td>0.5</td>
<td>0.03</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Both samples contained a background concentration of 800 mg Zn/kg sediment.

\textsuperscript{a} The pentachlorophenol was added a week before the addition of chloroform. PCP is pentachlorophenol. DCE is 1,2-dichloroethane. Inc is incubation time. HL is half-life.
Fig. 2. The effect of chlorpyrifos on the mineralization of chloroform. EC\textsubscript{50} = 4700 mg/kg; EC\textsubscript{10} = 1600 mg/kg.

toxic effects. Table 1, line 3, reflects an IC\textsubscript{10} of 1300 mg chlorpyrifos/kg sediment. This concentration is much higher than the maximal allowable concentration of 0.75 mg/kg derived from animal toxicity data (Denneman and Van Gestel, 1990).

The Effects of Cadmium and Mercury

Figure 3 indicates that addition of cadmium decreased the chloroform mineralization only at very high doses. The same holds for mercury (Fig. 4). This relatively low toxicity of cadmium and mercury might be attributed to the presence of sulfides in these anaerobic sediments which can cause precipitation. The highest reported concentrations of cadmium and mercury in Dutch river sediments are 185 and 155 mg/kg, respectively (Van Luin and Stortelder, 1990). Table 1 demonstrates that the IC\textsubscript{10} concentration of cadmium is 1300 mg Cd/kg. Therefore, no direct effect of the environmental cadmium concentrations is expected on the chloroform mineralization. For mercury the IC\textsubscript{10} is 90 mg Hg/kg which indicates that direct effects on the chloroform mineralization will be rare.

Fig. 3. The effect of cadmium on the mineralization of chloroform. EC\textsubscript{50} = 5200 mg/kg; EC\textsubscript{10} = 1700 mg/kg.
The Effect of Benzene

Figure 5 demonstrates that benzene is not very toxic for the chloroform mineralization in sediments. Biodegradation and sorption of benzene is slow in these sediments (Van Beelen and Van Keulen, 1990). Hence, benzene is bioavailable but indicates a high IC_{10} of 140 mg/kg (see Table 1). Benzene is present at concentrations below 0.1 μg/liter in the water of the river Rhine. Due to sorption it will have concentrations in the milligram per kilogram range in sediments (Slooff et al., 1988). Hence, no direct effects of benzene on the chloroform mineralization can be expected.

The Effect of 1,2-Dichloroethane

Figure 6 indicates that 1,2-dichloroethane inhibits the chloroform mineralization at very low doses. The concentrations are expressed in micrograms per kilogram. The IC_{10} of 0.07 mg/kg (see Table 1) is very low compared to the toxic effects of 1,2-dichloroethane to fish. The 96-hr LC_{50} of 1,2-dichloroethane is 480 mg/liter for the fish *Lepomis macrochirus* and 550 mg/liter for another fish named *Lepomis beryllina* (Dawson et al., 1975). The estimated global emission of this volatile compound is very high (Stringer, 1988). The octanol water partitioning coefficient of 1,2-dichlo-
Fig. 6. The effect of 1,2-dichloroethane on the mineralization of chloroform. The EC\textsubscript{50} = 0.66 mg/kg and the EC\textsubscript{10} = 0.09 mg/kg were derived from the carbon dioxide production curve.

roethane is 28 (Van Vlaardingen and Van Beelen, 1992). It can be calculated that sediment with 3% organic carbon and 0.07 mg 1,2-dichloroethane/kg is in equilibrium with 0.2 mg/liter using the Karickhoff equation (Karickhoff, 1981). Hence, this industrially very important compound is 3 orders of magnitude more toxic to the anaerobic microbial community which mineralizes chloroform, compared to fish. The concentration of 1,2-dichloroethane in water ranges from <0.06 to 10 μg/liter. Hence, direct effects of 1,2-dichloroethane on the mineralization of chloroform might be rare.

The Effect of Zinc Addition

Figure 7 demonstrates the effect of zinc added to the sediment coded G-mud-F on the mineralization of chloroform. The effect of zinc addition indicated a very large difference between the samples from 1987 and 1990. It seems that the sample G-mud-B from 1987 is less sensitive for pentachlorophenol or zinc than the samples taken later from the same location (see Table 1). According to the local authorities, the harbor was not dredged in the meantime. The large spatial variation which is often observed in sediments (Stemmer et al., 1990) might cause differences in the sorption.

Fig. 7. The effect of added zinc on the mineralization of chloroform in sediment G-mud-F which already contained 800 mg Zn/kg. EC\textsubscript{50} = 320 mg added Zn/kg dry sediment; EC\textsubscript{10} = 25 mg/kg.
of zinc. Another important factor is the precipitation of ZnS by the following reaction: 
\[ \text{Zn}^{2+} + \text{FeS}(s) \rightarrow \text{ZnS}(s) + \text{Fe}^{2+}. \]

It has been demonstrated that the amount of FeS measured as acid volatile sulfide (AVS) is a key factor in the toxicity of metals for marine and freshwater sediments. The distribution of AVS in intact sediment cores exhibits both spatial and temporal variation over the annual cycle (Di Toro et al., 1990). A partial aeration of the sediment by the activity of boats or strong currents might cause an oxidation of the AVS and, therefore, an increased sensitivity of the sediments for metals. The IC\textsubscript{50} of zinc addition on the mineralization of chloroform is surprisingly low since chemical analyses showed that the homogenized sediment samples G-mud-B and G-mud-C contained a background concentration of 800 mg Zn/kg dry sediment. Especially for the latter sample the very small addition of 37 mg/kg to the large amount of 800 mg zinc/kg present caused a clear effect. A small addition of 48 mg Zn/kg sediment had also an effect on the methane production in this sediment (Van Vlaardingen and Van Beelen, 1992).

It is possible that the additional zinc exceeds the amount of AVS present and causes a large increase in the bioavailability of zinc. Zinc concentrations in Dutch river sediments are 800 mg/kg on average with 90% of the samples showing a Zn content below 1800 mg/kg (Van Luin and Stortelder, 1990). Within 16 hr, about 31% of the zinc can be desorbed to a Chelex 100 cation exchange resin under aerobic conditions (Van de Meent et al., 1990). Under anaerobic conditions, however, only 0.4% of the zinc was desorbed. Hence, the bioavailability of zinc might increase rapidly when oxygen is added to the sediment. This might explain the high variability of the toxic effects of zinc on the microbial activity in different samples from the same site.

CONCLUSIONS

The IC\textsubscript{10} concentrations of added cadmium, chloropyrifos, benzene, mercury, or 1,2-dichloroethane were 1300, 1300, 140, 90, and 0.07 mg/kg dry sediment, respectively. Pentachlorophenol demonstrated an IC\textsubscript{10} of 15 or 150 mg/kg in different samples. For these compounds the sediment concentrations are too low to expect direct effects on the mineralization of chloroform. The toxic effect of 1,2-dichloroethane on the chloroform mineralization was 3 orders of magnitude larger than the effect on fish.

A partial inhibition of the mineralization of chloroform by the high concentrations of zinc present in Dutch river sediments cannot be excluded. Hence, the high concentration of zinc might cause prolonged persistence of organic pollutants in sediments.

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