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Effect of Methylene Chloride on Respiration and Electron Transport System (ETS) Activity in Freshwater Sediment

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Chlorinated hydrocarbons such as methylene chloride are widely used as a solvent for cellulose acetate, and in cleaning and degreasing fluids (Windholz, 1976). As a result of their extensive usage, they may be found in the environment (Brunner et al., 1980). Klecka (1982) stated that methylene chloride was generally considered non-biodegradable, since results using biochemical oxygen demand testing revealed negligible oxygen consumption over a 20-day period in the presence of methylene chloride. Brunner et al. (1980) reported on the capability of a facultative methylotrophic bacterium to use methylene chloride as a substrate. Rittmann and McCarty (1980) also reported that methylene chloride was utilized by sewage microorganisms. However, little information on the toxicity of methylene chloride in freshwater sediment is available. The present study reports on the effects of methylene chloride on microbial respiration, and electron transport system (ETS) activity in freshwater sediment.

MATERIALS AND METHODS

Sediment was collected in September 1983 from the surface 5 cm of a freshwater stream at Floradale, Ontario, Canada. Water samples were also collected at the same time in sterile polyethylene containers and immediately transported to the laboratory. Some characteristics of the sediment (Trevors, 1983) and water column have previously been described (Tam et al. 1981).

Sediment samples (10.0 g) were each placed in 50-mL Erlenmeyer flasks with 5 mL of stream water. All flasks used in respiration studies were sealed with serum stoppers (Suba Seal, Barnsley, England) and incubated statically in the dark at 20°C.

For 0_2 and $C0_2$ analysis, 0.2 mL of the gas phase from each serum-stoppered flask was withdrawn with a 1.0 mL syringe equipped with a gas-tight Mininert valve (Precision Sampling Corp., Baton Rouge, LA). A Gow-Mac 69-150 thermal conductivity gas chromatograph equipped with a 183 cm x 6 mm, 80/100 mesh Chromosorb 102 column, and a 91 cm x 6 mm, 60/80 mesh molecular sieve 5A column was used for analysis of $C0_2$ and 0_2, respectively. The carrier gas was

pure He at a flow rate of 21 and 52 mL min^{-1}, in the two columns, respectively. The detector was operated isothermally at 50°C with a bridge current of 150 mA. A detailed description of the method has been reported by Trevors (1983 b). All gas chromatographic data are reported as the average for triplicate flasks on a per g dry weight basis.

Electron transport system (ETS) activity was measured using the 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) reduction assay as described by Trevors (1983 a, 1984). In this investigation 1.0 g of sediment and 0.5 mL of stream water were incubated in a 16 x 150 mm test tube with a range of methylene chloride concentrations. Triplicate tubes were sacrificed periodically to determine the effect of methylene chloride on ETS activity, over extended periods of time. At each sample time, 0.3 mL of 0.4% filter sterilized INT was added to the sediment samples, which were further incubated for 1 h at 20°C in the dark. The INT-formazan was extracted with methanol and quantified spectrophotometrically at 480 nm using a Zeiss PM2A spectrophotometer and the procedure described by Trevors (1983a, 1984).

RESULTS AND DISCUSSION

Methylene chloride caused very different effects in the sediment samples depending upon the method used to measure activity. Table 1 clearly shows that methylene chloride had no significant toxic effect during a 1 hour enzymatic assay of ETS activity. Generally, a 1 hour assay is sufficient to obtain a representative measure of ETS activity. However, it does exclude the possibility that a toxic effect may be produced after a longer period of exposure, or a toxic effect is produced for a short duration, followed by a return to unperturbed levels of activity. To investigate the long-term effects of methylene chloride on ETS activity, sediment was treated with methylene chloride and assayed for activity over a 11-day period by sacrificing samples (Table 2). Methylene chloride caused a stimulation of ETS activity at day 1 and 4 when compared to the untreated controls. After 6 and 8 days, no significant inhibition or stimulation of activity was observed, whereas at day 11, a stimulation of activity was again observed. Moreover, ETS activity fluctuated between high and low values, both within and between the concentrations tested. The fluctuations displayed an erratic stimulatory effect. However, this may indicate that the total sediment stability is markedly altered when compared to the relatively stable ETS activity observed in the controls. Fluctuations in overall bioactivity may in fact represent an unstable situation which is just as detrimental as a significant inhibitory effect. In this respect, these fluctuations may be an undesirable environmental insult.

Oxygen uptake and CO_2 evolution have been used to assess microbial activity in a number of investigations (Klecka, 1982; Trevors, 1983b). Methylene chloride significantly inhibited CO_2 evolution in

the sediment. The effective concentration inhibiting CO_2 evolution by 50% (EC_{50}) after 7 days was estimated to be 11.7 µl/g (Table 3). Oxygen uptake measured in the same sediment samples treated with methylene chloride indicated that it was being used as a substrate, and stimulated ETS activity and therefore O_2 uptake. Because no inhibition was observed, an EC_{50} value could not be calculated. This can be supported by the findings of the ETS measurements, where increases in activity were also observed. However, these trends were not as definitive as the trends seen in the O_2 uptake measurements.

It appears that methylene chloride does have a definite inhibitory effect on CO_2 evolution, and causes highly variable fluctuations in sediment ETS activity. O_2 uptake was stimulated by similar concentrations ranging from 1.0 to 20 µl/g. The data clearly showed that any investigation of non-toxic or toxic effects of pollutants on environmental processes should not rely on any single measurement of microbial activity. Moreover, if conflicting results are obtained, they should be reported to assist in a more useful assessment of the environmental toxicity of chemicals under investigation.

Table 1. Effect of methylene chloride on ETS activity in freshwater sediment during a 1 hour assay

Methylene chloride (µl/g)	ETS activity[a] (µg INT-F/g)
0	161.6 ± 14.9
10.0	146.6 ± 11.2[b]
20.0	148.2 ± 24.4[b]
30.0	150.3 ± 19.4[b]
40.0	148.3 ± 12.9[b]
50.0	190.1 ± 16.9[b]

[a]Mean ± S.D. (n = 3)

[b]No significant difference from the control, as determined by a 2-tailed Student's t-test at P = 0.05.

Table 2. Effect of methylene chloride on ETS activity in sediment. Sediment treated with methylene chloride was incubated for the indicated period of time, and assayed for ETS activity for 1 hour.

Methylene chloride (µl/g)	Exposure time (days)	ETS activity[a] (INT-F µg/g)
Control (0)	1	32.7 ± 3.3
1		95.5 ± 30.4[b]
5		93.4 ± 13.8[b]
50		62.7 ± 18.1[b]
Control	4	55.1 ± 4.5
1		92.0 ± 21.0[b]
5		171.1 ± 44.9[b]
50		92.6 ± 3.5[b]
Control	6	78.2 ± 25.2
1		81.5 ± 45.5
5		135.2 ± 29.6
50		146.1 ± 79.5
Control	8	78.2 ± 63.3
1		122.0 ± 37.0
5		158.8 ± 41.6
50		79.5 ± 5.6
Control	11	32.0 ± 6.4
1		112.5 ± 5.5[b]
5		95.2 ± 23.1[b]
50		61.3 ± 18.8

[a]Mean ± S.D. (n = 3)
[b]Significantly different from the control, determined using a 2-tailed Student's t-test at P = 0.05.

Table 3. Effect of methylene chloride on CO_2 evolution in sediment. The EC_{50} (concentration inhibiting CO_2 evolution 50%)[c] was calculated using an Apple II plus microcomputer and an EC_{50} probit program based on the method described by Hubert (1980).

Methylene chloride (µl/g)	Incubation (days)	CO_2 evolution[a] (nmoles/g)
Control (0)	1	1111.1 ± 0
1.0		1037.0 ± 128.3
5.0		962.9 ± 128.3
10.0		755.5 ± 153.9[b]
20.0		814.7 ± 128.3[b]
Control	2	1777.7 ± 0
1.0		1629.5 ± 128.3
5.0		1259.2 ± 128.2[b]
10.0		999.9 ± 111.1[b]
20.0		925.9 ± 64.1[b]
Control	3	3043.8 ± 116.3
1.0		2784.6 ± 403.7
5.0		2121.7 ± 490.9[b]
10.0		1489.4 ± 346.3[b]
20.0		1259.2 ± 128.3[b]
Control	5	4443.9 ± 307.9
1.0		4710.5 ± 153.9
5.0		3732.9 ± 923.6
10.0		2221.9 ± 153.9[b]
20.0		1599.8 ± 102.3[b]
Control	7	5184.9 ± 256.6
1.0		5259.1 ± 128.3
5.0		4073.9 ± 256.6[b]
10.0		2962.8 ± 128.3[b]
20.0		1555.5 ± 222.2[b]

[a]Mean ± S.D. (n = 3)
[b]Significantly different from the control, determined using a 2-tailed Student's t-test at P = 0.05

[c]EC_{50} = 11.7 µl/g (calculated using data from 7 day analysis).

95% confidence limits for EC_{50} estimate 9.9; 13.8 µg/g

Table 4. Effect of methylene chloride on O_2 uptake in sediment

Methylene chloride (µl/g)	Incubation (days)	O_2 uptake[a] (µmoles/g)
Control (0)	1	5.9 ± 0.60
1.0		6.6 ± 0.59
5.0		9.7 ± 0.60[b]
10.0		19.4 ± 0.60[b]
20.0		23.0 ± 0.30[b]
Control	2	7.1 ± 0.57
1.0		8.4 ± 1.50
5.0		16.3 ± 1.00[b]
10.0		25.5 ± 0.56[b]
20.0		28.0 ± 0.97[b]
Control	3	8.8 ± 1.50
1.0		11.7 ± 0.51[b]
5.0		17.8 ± 1.40[b]
10.0		22.2 ± 0.51[b]
20.0		22.8 ± 1.50[b]
Control	5	16.9 ± 0.47
1.0		19.7 ± 0.47[b]
5.0		24.4 ± 0.95[b]
10.0		28.7 ± 0.46[b]
20.0		29.7 ± 0.63[b]
Control	7	18.2 ± 0.49
1.0		19.6 ± 0.85[b]
5.0		22.1 ± 1.70[b]
10.0		28.1 ± 0.85[b]
20.0		27.8 ± 0.98[b]

[a]Mean ± S.D. (n = 3)
[b]Significantly different from the control, determined using a 2-tailed Students t test at P = 0.05.

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