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Disappearance Rates of Chlorothalonil (TCIN) in the Aquatic Environment

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Chlorothalonil (TCIN) is a chlorinated isophthalonitrile fungicide with low water solubility. It is highly toxic to fish (Davies and White 1985) with 96h LC50 values in the range 10 - 30 µg/l, and is rapidly metabolised to glutathione conjugates (Davies 1985 a,b). It is released into streams at low total concentrations after agricultural spraying operations (Davies unpub. data). TCIN is biodegraded in temperate soils to its 4-hydroxy phenolic derivative, DAC3701, with a half-life of 2.5 - 3 months (Stallard and Wolfe 1967). DAC3701 is also produced by aqueous hydrolysis of TCIN (Szalkowski and Stallard 1976).

A series of experiments were performed using TCIN and ^{14}C-TCIN in aqueous solutions from different sources, at different temperatures and with different stream substrates, in order to evaluate the nature and rates of TCIN disappearance and the appearance of polar derivatives of TCIN (D_{TCIN}) in the aquatic environment.

MATERIALS AND METHODS

TCIN was purified by xylene extraction of technical grade Bravo® (Diamond Shamrock Corp.). After solvent removal at reduced pressure, the residue was recrystallised (3x) in acetone-water and dried in vacuo. ^{14}C-TCIN was prepared as described by Davies (1984), ≥ 98% radiochemical purity, 0.734 mCi/mmol. All solvents were redistilled A.R. grade.

Typical experimental procedure is as follows. Water (20 l), contained in glass aquaria housed in a constant temperature room, was inoculated with an acetone stock of TCIN or ^{14}C-TCIN to a TCIN concentration of 20 µg/l. At various time intervals, 240 ml water samples were extracted with 10 ml hexane by magnetic stirring for 20 min (96% efficiency by internal standard). The hexane fractions were dried (Na_2SO_4 anhyd) and analysed for TCIN by G.C. on a Perkin Elmer 881 gas chromatograph using 10% methane in argon as carrier, with 2% OV101/3%QF1(1:1) on a Gaschrom Q 100-120 mesh column at 200° C. Detection limit for TCIN = 0.001 µg/l on a Pye electron capture detector at 270°C. Hexane fractions were radioanalysed for ^{14}C-TCIN by counting 1 ml in 5ml Dimilume-30 on a Packard Prias-PL Tricarb scintillation counter.

The waters used were collected from North West Bay River, southern Tasmania, at two sites. Site 1 was in the stream headwaters (conductivity = 70 µS/cm, pH = 6.8), and site 2 was 30 km downstream in agricultural country (conductivity = 250 µS/cm, pH = 7.1). Dolerite cobbles for stream substrate studies were collected at site 2 from the same river. These were either collected wet with algal aufwuchs intact or cleaned with a brush on site then scrubbed under hot water and rinsed with 100% ethanol. Rock surface area, estimated by wrapping with plastic film (Gladwrap®) and weighing the cut shape, was 1000 ± 200 cm^2 per aquarium.

Test water from site 2 was used in order to examine rates of disappearance of TCIN from stream water. Two replicate tanks each were set up with still water; continuously aerated water; continuously aerated water with rock and attached aufwuchs, at each of 5 and 15° C. All tanks were inoculated to an initial TCIN concentration of 20 µg/l and the water sampled at regular intervals over a 75 hr period. Analysis of water for TCIN was by extraction and G.C.

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In order to examine the effect of temperature, stream substrate, water type and fish on disappearance of TCIN and the appearance of D_{TCIN}, duplicate tanks were set up as follows, with continuous aeration: distilled water only; site 1 water only; site 2 water only; rock and aufwuchs, site 2 water; clean rock, site 2 water; 2 Galaxias auratus (10 g/20l, unfed), site 2 water. Tanks were inoculated with ^{14}C-TCIN to an initial activity of 10^5 dpm/ml (20 µg/l). Water samples were taken at regular intervals over a 14 day period and analysed by extraction with hexane and radiocounting of both the hexane and water phases. Whole fish and algal aufwuchs were collected after 14 days, oven dried (60° C), weighed and digested in Soluene® prior to radioanalysis.

Exponential or parabolic curves were fitted by least mean squares (NWA STATPAK) to the concentration time series for each treatment. Mean curve constants and estimated half lives were derived for replicate treatments.

TCIN partitioning behaviour between water and non-filterable stream residue (NFR) and between water and n-octanol was examined. TCIN was released into an upper reach of Tucker's Rivulet, a small agricultural stream (0.05 cumec flow, 0.27 m^2 cross-sectional area, 0.424 m/s mean current speed) whose lower reaches were contaminated with TCIN from spraying operations (Davies unpub. data). Three replicate water samples (250 ml) were taken 0.67 km downstream at different times after TCIN release, in acetone rinsed glass bottles, and filtered through dried (100° C) pre-weighed glass filters. The filters were dried (60° C), re-weighed, and the filtered material on the upper surface was extracted (60 min) with acetonitrile (5 ml). TCIN was extracted for analysis from the filtered water with hexane as described above.

^{14}C-TCIN (0.7 mg) and TCIN (20 mg) was stirred (48 h) between redistilled n-octanol (2 ml) and 0.01M phosphate buffer (2 ml, pH 7.0) at 20°C. The two layers were separated, filtered (glass-fibre) and aliquots were radio-counted. The test was performed in triplicate.

RESULTS AND DISCUSSION

Time constants and half-lives for TCIN disappearance are shown in Table 1. Time constants and half-lives for production of D_{TCIN}, calculated from sample aqueous counts after extraction, are shown in Table 2. TCIN levels in aufwuchs algae and fish tissue after 14 days exposure are shown in Table 4.

TCIN was highly associated with suspended material (as non-filterable residue or NFR) in stream water (Table 4). The log octanol-water partition coefficent (log $P_{o/w}$) was determined as 4.38 (SD 0.12).

Loss of TCIN was slow in still water at 15° C, with 42% remaining 75 hr after inoculation. TCIN levels dropped to 50% in 101 hr when the water was aerated, indicating enhanced loss by volatilization and/or surface adsorption. The addition of rock with attached algal aufwuchs caused a still greater enhancement of TCIN loss so that only 10% remained at 2 days, with none detected at 10 days.

Loss of TCIN at 5°C was markedly slower than at 15°C. Comparison of loss rates gave a Q_{10} value of 1.8, in agreement with values expected for biochemical processes with enzyme activation energies around 10 kcal/mole (Larson et al. 1981).

Despite the fact that no significant differences in TCIN disappearance rates were caused by water type, D_{TCIN} appearance rates were affected, increasing in the order: Distilled Water (1) < Site 2 (3.3) ≤ Site 1 (3.5). This indicates that D_{TCIN} appearance is affected by microfloral and/or solute concentrations.

Table 1 Disappearance half-life, time constant and loss of TCIN in stream water. {Regressions of the form $y = a.e^{bt}$ (*) and $y = a.t^{b}$ (**), with t in hr, y in µg/l. $N \geq 7$, $p < 0.05$ all regressions. + indicates use of TCIN, all other tests performed using ^{14}C-TCIN}

Conditions	Water Origin	Temp (°C)	Half-life (hr)	Time constant b (x 10^{-2})	Loss (%) (by x hrs)
Still water+	Site 2	5	150.0	-0.462*	25 (75)
Still water+	Site 2	15	80.0	-0.866*	42 (75)
Aerated water, rock+aufwuchs.+	Site 2	5	13.9	-4.987*	96 (75)
Aerated water, rock+aufwuchs.+	Site 2	15	7.7	-9.002*	≥99 (75)
Aerated water	Distilled	15	106.3	-0.652*	94 (336)
Aerated water	Site 2	15	101.3	-0.684*	94 (336)
Aerated water	Site 3	15	107.3	-0.646*	94 (336)
Aerated water, G. auratus.	Site 2	15	4.3	-47.9 **	96 (336)
Aerated water, rock+aufwuchs.	Site 2	15	4.4	-46.7 **	96 (336)
Aerated water, rock surface.	Site 2	15	90.6	-0.765*	95 (336)

The algal aufwuchs layer on stream cobbles played a dominant role in TCIN removal from the water column. Removal of aufwuchs from cobbles caused a decrease by a factor of 61 in TCIN loss rates. The rate of TCIN loss was not significantly enhanced by the presence of a sterile dolerite rock surface in aquaria. Benthic algae have been found to concentrate highly chlorinated dieldrin by up to 30000 times in laboratory streams contaminated at 0.05 - 7 µg/l over four months (Rose and McIntire, 1970).

Table 2 Appearance half-life, time constant and appearance of TCIN polar metabolites, D_{TCIN}, in stream water. {Regressions of the form $y = 25 - a.e^{bt}$, with t in hr, y in µg/l. $N \geq 7$, $p < 0.05$ all regressions.}

Conditions	Water Origin	Half-life (hr)	Time constant b (x 10^{-2})	Present (%) by 14 days
Aerated water	Distilled	567.3	-0.131	84.1
Aerated water	Site 2	172.1	-0.406	85.2
Aerated water	Site 3	161.3	-0.431	80.2
Aerated water, G. auratus	Site 2	55.9	-1.240	90.1
Aerated water, rock+aufwuchs	Site 2	237.4	-0.292	81.5
Aerated water, rock surface	Site 2	163.9	-0.423	91.4

Analysis of the aufwuchs material demonstrated a concentration factor for TCIN of 270 times. However, this represented only 9.5% of the initial TCIN dose. It appears therefore that stream-bed aufwuchs increased the rate of TCIN removal by enhancing the conversion rate to

polar metabolites. In lotic waters, the ability of the aufwuchs to concentrate TCIN may be more important than its ability to metabolize it, due to the unequilibrated state of stream flow. The average initial rate of TCIN degradation was estimated at 3.4 µg/h/g wet weight algal aufwuchs, or 64 µg/h/m^2 rock surface, at 20 µg/l TCIN concentration.

The rate of ^{14}C-TCIN loss from solution was enhanced by a factor of 25 times when G. auratus was present at 10 g/20l. The rate of appearance of D_{TCIN} increased by three times. Fish can readily take up TCIN from water (Davies 1985a), and the difference in rates here indicates a substantial accumulation. The average initial uptake rate was 0.77 µg/h/g fish tissue, 5 times lower than for aufwuchs algae.

Despite the highly chlorinated nature of TCIN, it is readily biodegraded in stream water, in contrast to other chlorinated hydrocarbons such as kepone and DDT (Chakrabarty 1982). The nitrile functional groups.

Table 3 ^{14}C-TCIN residues present in aufwuchs algae and fish tissue after 14 day static exposure to 20 µg/l ^{14}C-TCIN.

	Aufwuchs density	[^{14}C-TCIN], µg/g wet wt	dry wt	% of total tank dose	BCF (wet wt)
Aufwuchs	36.2 - 41.5 mg dry wt/100cm^2 rock surface	5.41	27.05	9.5	271
Fish tissue	-	0.35	2.5	3.4	18

direct nucleophilic attack such as hydrolysis to the 4 and 6 chlorine positions (Vincent and Sisler 1968; Davies 1985b). Insertion of chlorine on the aromatic nucleus of a hydrocarbon slows the rate of biological attack by electronegative deactivation (Chakrabarty 1982), and it has been shown to inhibit the primary oxidation of chlorobiphenyls by microorganisms. Such oxidative processes are not likely for TCIN due to complete aryl substitution, while attack by nucleophilic species is predominant. It is highly likely that the principle degradative product is the polar 4-hydroxy trichloroisophthalonitrile, DAC3701, except in the presence of fish where mercapturate metabolites are more likely (Davies 1985b).

Table 4 TCIN on suspended matter (NFR) in environmentally exposed stream water (N = 3 at each concentration).

[TCIN], µg/100g in water (total)	on NFR (X10^6)	[NFR] (mg/l)	% TCIN on NFR
2.3	2.17	20.9	95.2
27.0	10.73	13.8	84.5
138.2	18.35	13.6	64.4

TCIN is readily biodegraded at low concentrations and cannot be regarded as a persistant pollutant. It is unlikely that biodegradation will play a major role in the fate of TCIN in moderate to fast flowing streams where volatilization and stripping by adsorption are liable to be dominant factors.

Analysis of NFR in samples of stream water contaminated with TCIN (Table 4) demonstrated significant binding to suspended material with an average log partition coefficient (log $P_{sm/w}$) of 5.695, and with an average of 81% of the total TCIN being bound to suspended matter. Some dependence of binding on concentrations of TCIN and suspended matter was observed. Such high affinity with suspended material is to be expected in the light of known relationships between the water solubilities and the organic matter-water partition coefficients for organic compounds (Chiou 1981). The relationship given by Chiou (1981) between the octanol-water partition coefficent ($P_{o/w}$) and the water solubility predicts a value log $P_{o/w}$ value of 4.97, agreeing well with the value of 4.38 ± 0.12 obtained here.

TCIN does not behave like other chlorinated hydrocarbons in that the parent compound is not bioaccumulated to any appreciable extent in fish tissue (Davies unpub. data). Despite close similarities in physical behaviour, the directing effect of the nitrile groups has a major influence on the biochemical fate of TCIN.

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