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Competition of Fate Processes in the Bioconcentration of Lindane

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Lindane, the gamma (γ) -isomer of hexachlorocyclohexane is one of the few hydrocarbon insecticides still widely used for agricultural and horticultural purposes. Use of technical grade lindane has increased gradually over the last two decades, averaging only 16 t in 1960 and 100 t in 1976 (Statistics Canada 1960-1980). Lindane can leach slowly from agricultural soil into streams or ground water. Typical levels of lindane in surface waters of Alberta, Canada, are 0.001 µg/L (Environment Canada 1976-1981). Higher concentrations might occur in drainage ditches and streams for short periods in spring and summer after intensive agricultural use. Peak values of up to 10 µg/L of lindane in surface waters of England and Wales have been reported (Tooby and Durbin 1975).

The bioaccumulation and toxicity of lindane to aquatic organisms have been reported in the literature from laboratory studies using flow-through systems with continuous spiking of lindane to maintain its concentration in water constant. However, the design of these systems prevents the determination of the significance of fate processes such as sorption and volatilization by saturation of the absorbing surfaces and by constant mass flow of lindane to replenish the loss. Fate processes are predominant in nature and are likely to compete with bioconcentration and hence influence the toxicity of lindane to fish. Thus the effect of such processes on the residence time of lindane and its concentration in water have not been studied so far. The purpose of this study was (i) to investigate the role of sorption by natural and synthetic materials as well as volatilization on the residence time and concentration of lindane in water; and (ii) to predict and compare with literature, the values of partition coefficients, bioconcentration factor, volatilization rate constant and the half-life for volatilizational loss from water. Studies were conducted in a laboratory tank system containing river water and bottom sediments in which rainbow trout (Salmo gairdneri Richardson) eggs and fry were exposed to low concentrations of lindane in water for a period of 5 weeks. The embryo-larval stage is one of the most sensitive in the life cycle of fish (MacDonald 1979) and its use has been suggested (McKim 1977) in establishing water quality criteria.

METHODS AND MATERIALS

The experimental exposure system consisted of:
(i) nylon mesh (5-6 mesh/cm) baskets (9w x 10ℓ x 14.5h cm) to contain the eggs and fry, (three baskets per chamber);
(ii) small plexi-glass (9L capacity) exposure chambers (13w x 47ℓ x 21h cm) to hold the sediment and water, ten chambers (6 for three lindane concentrations (a,b,c) in duplicate and 4 for controls), and
(iii) a temperature-controlled water tank (51cm w x 2.4m ℓ x 30.5 cm h) to maintain the exposure chambers at 10 ± 0.1°C.

The baskets were suspended in a row in each exposure chamber with the bottom of the baskets about 2 cm above the surface of the sediments (Fig.1). The chambers were held in the temperature controlled tank. Water from each exposure chamber was drained into a separate reservoir (5L) and recycled back at a flow rate of 180 ml/min. This eliminated any cross-contamination of lindane between chambers. Water in the chamber was agitated by bubbling air (0.5 - 1.0 L/min.) through a Hagen bubble wand placed along the length of the chamber. A strip of plexi-glass diverted a portion of the rising water across the surface of the eggs. Illumination was provided by the cool-white flourescent light and incandescent bulbs simulated a 15-min. dawn or dusk light intensity change. A constant 12 h light-, 12 h dark-period was controlled by an automatic timer. The tank was sealed with styrofoam on the sides to minimize temperature fluctuation and the chambers were also screened with nylon mesh to reduce the light intensity in the baskets to 21.5 lux units.

Surface sediment samples were obtained from the Vermilion River at a site approximately 5 km south of Vegreville, Alberta (latitude 53°27'N, longitude 112°4'W). The bed sediment was analyzed to be a mixture of silt and sand >250 μm(12%), 250-150 μm(22%), 150-63μm (33%) and <63 μm(33%). The sediment used in this study contained 60% moisture, 4.2% low temperature ashable (250°C for 3h) organic matter and 8.6% high temperature ashable (600°C overnight) organic matter. The bed sediment was analyzed and found to be free from lindane and other major contaminants (<0.1 μg/kg). Municipally treated river water was used after passing it through ion-exchange cartridges and activated carbon cartridge. Eyed rainbow trout eggs supplied by the Mount Lassen Trout Farms, Red Bluff, California, were used. The eggs were about 3 weeks old when received and the eggs without eyes and damaged or dead eggs were discarded.

Commercial grade γ -BHC (Sigma Chemical) was used to prepare a stock lindane solution of 800 μg/L in acetone. It was diluted 1:600 with river water for experimental use. Lindane was difficult to dissolve and proper care in terms of thorough mixing for about 20 h was devoted to ensure the solubility of lindane. Each exposure chamber contained 5.5 L of the river water and 1 kg(wet weight) of sediment covering an area of 611 cm^2 in each chamber. Lindane was added to duplicate chambers in amounts of (a) 700, (b) 350, and (c) 35 μg per chamber, as follows:
Sediments were added to 5.5 L of river water and stirred vigorously. Lindane stock solution was added to the sediment suspension in five

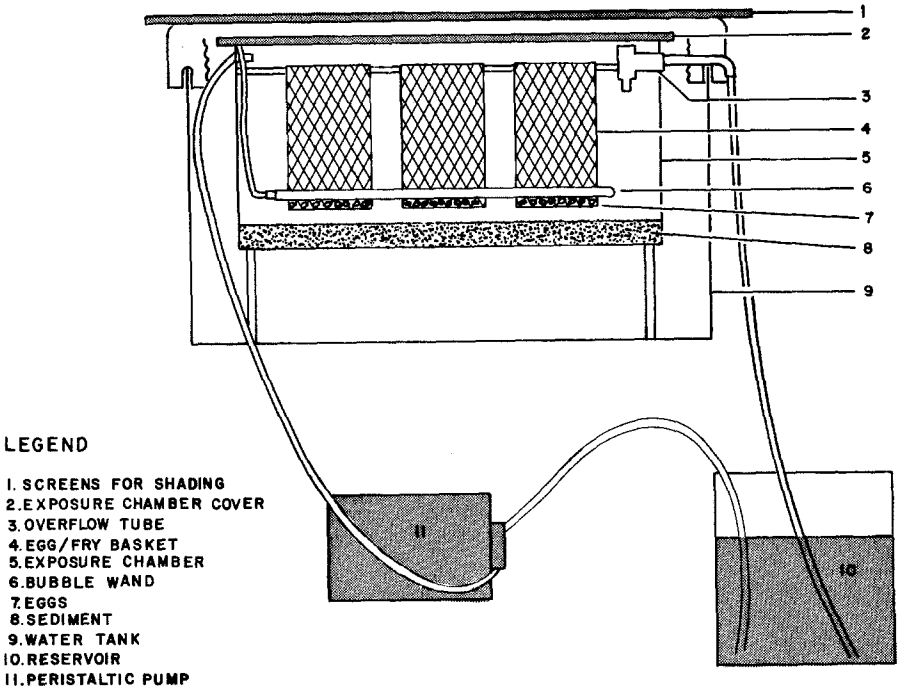

FIGURE 1. EXPOSURE SYSTEM FOR RAINBOW TROUT EGGS AND FRY.

100 ml batches over a period of 5 h. The sediments were stirred during each addition and at 20 and 40 min. intervals between the additions. The entire system was left undisturbed for 4 days to allow the finely divided suspended solids to settle out. More river water was added to bring the final volume to 13L and circulation was begun. Four control chambers without lindane were setup following the same procedures. Seven days after the addition of lindane, 100 eyed rainbow trout eggs were placed in each basket (300 eggs/chamber). Care was taken to protect the eggs from light and temperature shock. Eggs at all lindane treatments hatched between 7 to 14 days in the basket and the fry continued to be exposed to lindane.

The various partition coefficients were calculated using the regression equations relevant to the present system and the chemical tested, from the literature (Kenaga and Goring 1980; Lymen et al. 1982). These binary regression equations correlate between water solubility(WS), soil sorption coefficient(k_{OC}), octanol-water partition coefficient(k_{OW}), and bioconcentration in fish(BCF) (Fig.2).

Water samples taken at 1,4,7,15 and 33 days, sediments sampled on day 14, eggs on day 7 and fry on day 27 were analyzed by gas chro-

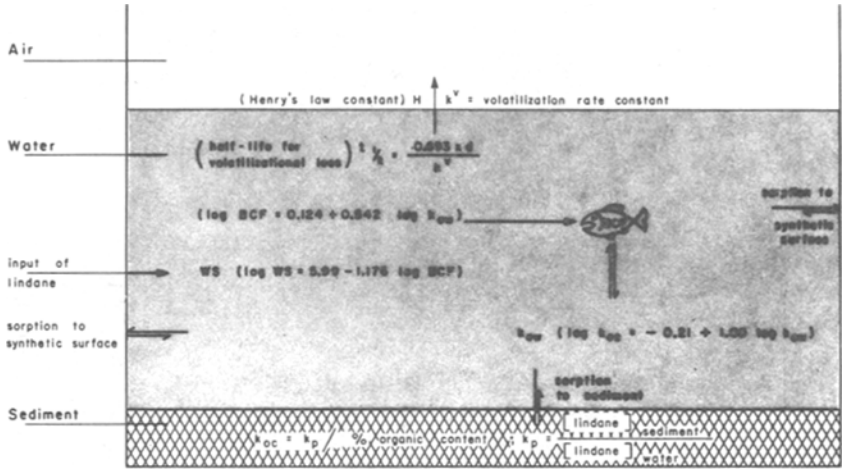

Figure 2. Partitioning of lindane in an aquatic system.

motagraphy (Environment Canada 1974) for γ- and α-lindane (Table 1). The precision and accuracy of the analytical method was (i) 0.63 ± 0.04 (0.69), (ii) 2.61 ± 0.05 (2.68), and (iii) 9.91 ± 0.04 (10.0); all values in µg/L and in each set, the mean value, reproducibility and the calculated value in brackets are given. Every batch of samples for analysis included not less than 20% quality control samples. A simple suction device was used to sample sediments from chambers to avoid mixing of sediment. Water above the sediment layer was siphoned off before sampling sediment. Eggs and fry were kept frozen until analysis.

RESULTS AND DISCUSSION

All of the water quality parameters except metals (<0.001 mg/L) and pH (8.1), increased over the experimental period, possibly from the sediments reaching an equilibrium with the recycling river water. Concentrations of lindane in the water column of all exposure chambers were <0.6 µg/L at the beginning of the exposure period and gradually declined thereafter (Table 1). Concentration of lindane in sediments was 100-300 times higher than those in water. Highest concentrations in sediments (up to 77 µg/kg) were found in chambers spiked with the highest amount of lindane (700 µg) (Table 1).

The average bioconcentration factor was 319(142.6/0.29; 64/0.24; 50.3/0.19; 59.1/0.19; 8.9/0.03 and 8.5/0.03, where the numerator is the concentration of lindane (average of two values, Table 1) (µg/kg) in fry and the denominator is lindane in water (µg/L), for the six exposure chambers on day 27. The actual biomass (fish) in each chamber was approximately 30 g only on day 27. More than 90% of lindane present in the system was sorbed to the sediment (1 kg wet weight in each exposure chamber). Relatively high sorption of lindane by sediment in the system left low amounts of lindane in the water column (0.1 - 0.6 µg/L). These values are much lower

Table I. Concentration of lindane in water, sediments and fish.

Concentration of lindane	day 1	4	7	15	33
Water, µg L^{-1}					
(700) #1	0.5 - 0.6	0.4	0.2 - 0.3	0.2 - 0.3	0.1
#2	0.4 - 0.6	0.4	0.2	0.1 - 0.3	0.3
(350) #1	0.5 - 0.6	0.1 - 0.2	<0.1	0.1 - 0.2	0.2
#2	0.5	0.2 - 0.3	0.2	0.2	0.1
(35) #1	<0.1	<0.1 - 0.2	<0.1	<0.1	<0.1
#2	<0.1	<0.1	<0.1	<0.1	<0.1

Sediment, µg.Kg^{-1}	day 14
(700) #1	47.2, 49.8
#2	55.6, 76.7
(350) #1	38.7, 41.7
#2	19.1, 18.5
(35) #1	10.7, 1.8
#2	<0.6, 11.6

Fish, µg.Kg^{-1}	day 7 eggs	day 27 fry
(700) #1	107.2, 95.8	160.6, 124.5
#2	66.8, 60.8	41.5, 86.4
(350) #1	48.0, 54.7	39.1, 61.5
#2	48.4, 41.1	64.3, 53.9
(35) #1	7.0, 6.9	8.5, 9.3
#2	6.2, 6.7	5.3, 11.8

*The value in brackets is the total amount of lindane added to each exposure chamber containing sediment (1 kg wet weight), water (13 L) and biota (~15 g initially as eggs and later ~30 g as fish fry).

than the lethal concentration reported for lindane in the literature (Tooby and Durbin 1975; Macek et al. 1976). The aqueous concentrations of lindane (0.2 - 0.6 µg/L) was found to be not acutely lethal to fish but fry exposed to such concentrations for a longer period developed symptoms of sublethal toxicity. These symptoms could be due to direct or indirect effect of build-up ammonia (mostly ionized), and other fish metabolites in addition to lindane. Studies (Tooby and Durbin 1975; Hansen 1980) have shown that lindane in lethal concentrations cause hypersensitivity in fish but behavioural changes at low concentrations of lindane have not been reported. The observed lethargism (as evidenced by the difference between the controls and experiment) in fry at concentrations of lindane used in this study could possibly lead to decreased food intake and increased predation. The data indicate that fertilized eggs accumulate lindane in significant amounts and similar levels

are found in sac fry exposed to very low concentrations. This could result from continued uptake and slow depuration of lindane from fish. Goldeye was shown to clear 90% of the accumulated lindane within a 2 day depuration period in control water (Gakstatter and Weiss 1967) whereas rainbow trout retained 35% of the accumulated lindane even after 19 days of recovery.

In this study, sorption of lindane to sediment as well as to the surfaces of the walls of the exposure chamber was investigated. The duplicated tests show almost 30 times greater sorption by plexi-glass container than glass surface for a 10-day period. At 15µg/L of lindane in water, up to 88% could be sorbed by the plexi-glass walls. Tests on glass containers (results not shown) revealed a considerable loss (65%) of lindane over a period of ten days due to volatilization with minimal sorption (3%) in glass container. Lindane is more volatile than most organochlorine pesticides (vapor pressure of 9.4×10^{-6} mm of Hg at 25° C, Martin 1972). There is a good agreement between the calculated, experimental and literature values of the paramaters;

Parameter	Expt.Value log	Calc.Value log	Lit.Value log	Reference
K_{oc}	3.52	--	3.30	Wahid and Sethunathan 1979.
			3.57	Karickhoff 1981.
K_{ow}	--	3.73	3.72	Kurihara et al.1973.
BCF	2.50	2.15	2.51	Macek et al. 1976.

Theoretical BCF values, using lindane solubility data (assuming the absence of other competing fate processes) are calculated to be higher than the experimental value by 10^3 fold. Kenaga (1980) reported that when 37 experimental values of BCF were compared with BCF values calculated from WS (water solubility) or K_{oc} equations, 87% fell within one order of magnitude and 100% within two orders. He concluded that unexpectedly high BCF values calculated from the equations may be due to poor water solubility or soil absorption data. Obviously, competing fate processes such as volatilization and sorption to surfaces of the container walls in addition to sorption to sediments have depleted lindane from the water column. This results in lower amount of lindane in water for bioconcentration by fish. This does not alter the BCF value but the actual amount accumulated. Thus competing processes could be beneficial in reducing the exposure concentration of the chemical for accumulation by biota in nature. However, these processes must be quantitated in order to reach the desired exposure concentration in toxicity assessment studies. Compounds having a K_{oc} value 1000 (such as lindane) will be strongly bound to organic matter in soil

Table 2. Henry's law constant, volatilization rate constant (k^V) from water and the half-life ($t_{\frac{1}{2}}$) for volatilizational loss from water for lindane.

Parameter	Equation used	Calc. Value	Lit. Value
H, Henry's law constant	$H = \frac{vP(\text{mm Hg}) \times M.W. \times 16.04}{T_k \times S}$ (a) where vP = Vapor pressure MW = Molecular weight T_k = Temperature in ° Kelvin and S = Solubility in water in μg mL^{-1}	0.202*	-
k^V, volatilization rate constant from water	$k^V = \frac{1}{d[3.1407\times10^{-4}\sqrt{MW}+(2.0413\times10^{-4}\times T_k \times S\times\sqrt{MW}/vP)]}$ (a) where d = Depth in cm MW = Molecular weight T_k = Temperature in ° Kelvin S = Aqueous solubility (moles L^{-1}) at T°K vP = Vapor pressure (mm Hg) at T°K	0.365* cm day^{-1}	0.240(b) cm day^{-1}
$t_{\frac{1}{2}}$, half life for volatilizational loss from water	$t_{\frac{1}{2}} = \frac{0.693 \times d}{k^V}$ where d = depth in cm and k^V = rate constant for volatilization of lindane from water	1.9 days (for 1 cm depth) 199 days (1 m depth)	2.89 days(c) (1 cm depth) 289 days (1 m depth)

(a) Roberts et al. (1981); (b) NRCC 18574 (1981); (c) Mackay and Wolkoff (1973).

*vapor pressure of 9.4×10^{-6} mm of Hg and aqueous solubility of 7.3 μg mL^{-1} for lindane at 25°C were used.

and are considered immobile (Kenaga 1980). The main route to surface waters will probably be through washing down of soil particles due to heavy rain or during spring thawing of snow and ice.

The calculated Henry's law constant, of 0.202 ($atm.m^3/g.mol$ units) (Table 2) indicates the dominance of the liquid phase resistance to lindane and thereby favouring partitioning of lindane in the vapor phase. For solutes of high H > 0.001($atm.m^3/g.mol.$ units), the liquid phase resistance dominates and for solutes of low H, < 0.0001 such as SO_2, the gas phase resistance dominates (Mackay et al. 1980). The volatility of organic chemicals has been classified according to Henry's Constant, H (EPA 1975). According to this scheme, chemicals having, H > 0.01 are readily lost from water surfaces; H = 0.01-0.00001 are moderately volatile and H < 0.00001 are non-volatile.

The calculated values of k^V, volatilizational rate constant, and $t_{1/2}$, half-life for volatilizational loss from water, are in reasonable agreement with the literature values, considering the widely ranging vapor pressure and aqueous solubility values for lindane reported in the literature. Use of higher values for vapor pressure and lower values for aqueous solubility would yield lower $t_{1/2}$ values. The experimental $t_{1/2}$ for volatilizational loss for lindane was about 5 days under moderate mixing conditions. The percent recovery of lindane from water, sediment and biota is given in Table 3. Using the experimental data and the calculated constants

Table 3. Percent recovery of lindane from exposure experiments

Spiked lindane concentration (µg)	Amount (µg) of lindane recovered from Sediment	Water	Biota	Total lindane (µg) in the three analyzed compartments	% recovery of lindane
35	10.7	1.3	0.14	12.14	35
35	11.6	1.3	0.14	13.04	37
350	40.2	1.95	1.03	43.18	12
350	18.8	2.60	0.98	22.38	6
700	66.2	2.6	2.05	70.85	10
700	48.5	3.25	1.38	53.13	8

for the fate processes, the percent distribution of lindane (total concentration, 350 µg) in the system would be as given in Table 4. The unaccounted lindane in the mass balance in the experimental system was ascribed to sorption on the plexi-glass walls as the most probable location (described earlier). Isomerization of lindane to α-BHC was found to be insignificant. Fate processes should be considered in designing aquatic toxicity experiments in order to reach the actual exposure concentration of the test chemical close to the desired exposure concentration.

Table 4. Percent distribution of lindane in the system

Water (13L)	Sediment (1 kg)	Biota (30g)	Walls of plexiglass chamber (calculated by difference)	Air (water depth = 15.5 cm)	Loss due to Isomerization	Loss due to Photo degradation
0.6	11.5	0.3	31	56.5	N.D.	N.D.

N.D. = Non Detectable

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