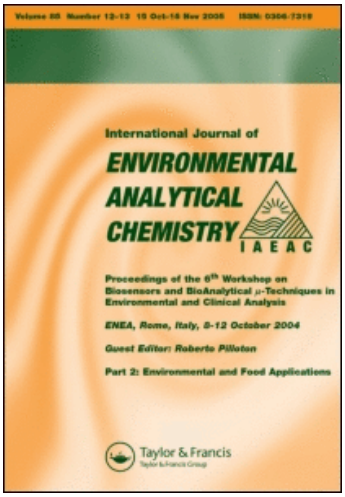

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Binding of Gamma-Hexachlorocyclohexane with Humic Acid and Chlorophyll in Aquatic Environment in Relation to Ecotoxicity

V. Misra[a]; S. D. Pandey[a]; P. N. Viswanathan[a]
[a] Ecotoxicology Section, Industrial Toxicology Research Centre, U.P., Lucknow, India

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BINDING OF GAMMA-HEXACHLOROCYCLOHEXANE WITH HUMIC ACID AND CHLOROPHYLL IN AQUATIC ENVIRONMENT IN RELATION TO ECOTOXICITY

V. MISRA*, S. D. PANDEY and P. N. VISWANATHAN

Ecotoxicology Section, Industrial Toxicology Research Centre, P. Box No. 80, M. G. Marg, Lucknow-226001, (U. P.) India

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The feasibility of using fluorescence quenching studies as a model for environmental dynamics was explored in a sand-water-macrophyte experimental system. The influence of humic acid (HA) on the release of gamma-HCH from sand into water and the macrophyte lemna was studied by fluorescence in the presence and absence of a detergent, linear alkylbenzenesulphonate (LAS). Gamma-HCH was found to enhance the fluorescence of humic acid whereas it quenched the fluorescence of chlorophyll. LAS showed an additive effect through solubilization. The consequences of HCH-humic interaction in relation to detoxification/potentiation effects in ecosystems are discussed.

KEY WORDS: Humic acid (HA), Gamma-hexachlorocyclohexane, chlorophyll, fluorescence, linear alkylbenzenesulphonate (LAS).

INTRODUCTION

Pesticides applied for agricultural and disease control purposes on crops and soil reach the aquatic environment through soil runoff, erosion, and leaching, eventually finding their way into sediments[1]. Sediments reflect the biological, chemical and physical conditions of a water body due to their complex role in deposition, release and distribution of many hydrophobic chemicals[2,3]. Sediments with higher concentrations of total organic carbon (TOC) have a greater capacity to absorb non-polar organic compounds, thereby reducing the toxicity[4]. Humic substances are found in natural surface waters, soils and sediments in both soluble and insoluble forms. Thus pesticide-humic interactions may alter the toxicity of agricultural chemicals. Humic acid can absorb solar UV radiations by virtue of the presence of ketonic and quinoid functional group in it and transfer energy to pesticides in the environment[5]. Apart from this, many environmental chemicals may act as quenchers in energy transfer processes and exist both in sand and in suspended phases.

* Author to whom correspondence should be addressed.

A number of authors have demonstrated that hydrophobic pollutants could bind to dissolved humic materials which could significantly affect the environmental behaviour of hydrophobic organic compounds[6]. Abdul *et al.*[7] used a humic acid solution to remove organic contaminants from hydrogeologic systems. Sediments also act as a modifying factor in pesticide-algae interactions[8]. Thus the dynamic nature of humic substances in ecosystems and the distribution and total mass of pollutants in ecosystems could be governed *inter alia* by pollutant-humus interaction. Recently, considerable interest has been shown in the design of experiments using duckweed (*Lemna minor*). Duckweed is a common floating macrophyte in fresh water has an excellent potential for use in ecotoxicological studies due to its sensitivity, small size, rapid growth, vegetative reproduction and shorter life cycle than millet and cabbage[9]. Therefore, a composite system consisting of sand coated with gamma-HCH, humic acid dissolved in water, and the duckweed was developed to study the release of gamma-HCH from the gamma-HCH coated sand in water containing humic acid and its effect on the fluorescence of humic acid. The role of humic acid in detoxification of gamma-HCH after UVB irradiation was also studied. In addition to humic acid, the chlorophyll of *Lemna* was also investigated to see whether it also is a site of binding of pollutants in ecosystems. Along with pesticides, the effect of the synthetic detergent (LAS) was also tested for its ecological effect *per se*[10–12], and its influence on the environmental kinetics and dynamics of other pollutants[13,14].

EXPERIMENTAL

Lemna culture

Axenic culture of duckweed (*Lemna minor, L.*) was obtained from the collection maintained by the Dept. of Botany, Lucknow University, Lucknow and vegetatively propagated by successive subculturing in the laboratory for the past five years under aseptic conditions[15]. Optimal growth conditions in modified Hoagland's solution was 7 days in continuous fluorescent light (Philips) 200 mole m^{-2} s^{-1} at 25 ± 0.5°C[16].

Chemicals

Gamma-HCH (99% purity) was purchased from Sigma Chemical Company, USA. Linear alkylbenzenesulphonate (LAS) of chain length C-13, procured from Indian Petrochemicals Ltd. (Baroda), was a commercial sample of acid slurry to be used in detergent powder and the purpose was to test the various factor influencing the aquatic ecotoxicity of the common water pollutants. Pesticide reference standards of Reidel-de Haen (West Germany) were a gift of RIVM (Netherlands). Other chemicals used in the experiment were from British Drug House (England) and E. Merck (Germany). Humic acid (sodium salt) from Aldrich Chemical Company was used in all studies.

Preparation of coated sand

Pure acid washed, calcinated sea sand (E. Merck) with particle size of 0.1–0.3 mm was used as simulated sediment by incorporating known amounts of the desired organics for

environmental dynamics studies. The sand was coated homogeneously with gamma-HCH (500 μg/g) by continuous shaking with desired amount in n-hexane. The gamma-HCH level was up to the saturation limit. The solvent was evaporated to dryness under controlled conditions (temperature and pressure) to avoid vaporization of gamma-HCH. In order to account for any evaporative loss, the HCH content of soil was analysed prior to the experiment.

HCH-humic acid interaction

In experimental systems, 10 g of loosely packed gamma-HCH coated sand (2 cm height) were taken in twelve 100 ml conical flasks. The experiments were performed in three sets. Each set consisting of four flasks, one each for 24, 48, 72 and 96 h. The first set comprised one with gamma-HCH coated sand with 30 ml, humic acid (50 mg/l), the sand with gamma-HCH coated sand with humic acid and LAS (5 μg/g), and the third (control) containing humic acid (30 ml) and sand without gamma-HCH. From each set at 24, 48, 72 and 96 h 5.0 ml of supernatant (medium) was taken from each flask for fluorescence measurement of humic acid.

To see the effect of UVB irradiation on the binding of humic acid and gamma-HCH, 16 beakers of 100 ml capacity were divided in two groups (control and experimental) of eight beakers in each. In the control, a layer of 10.0 g of pure sand was evenly spread in each of the eight beakers to a height of 2 cm and 50 ml of 10 μg humic acid was added. For the experiment 10.0 g of gamma-HCH coated sand (500 μg/g) was used. All the beakers were irradiated with UVB (sun lamp) light at room temperature up to 4.0 h. At the interval of 30 min, one beaker from the control group and an other from the experimental group were removed and the fluorescence and OD of humic acid was measured. The irridance of light was measured by Radiometer RMX 3W Vilber Lourmet (USP). The dose of irradiation was from 3.8 to 30.9 J (Joules). The height of the UV tube was 15.0 cm, length 135.5 cm and width, 11.5 cm.

To test the effect of various concentrations of gamma-HCH and detergent alone and in combination on the fluorescence of humic acid, three systems were developed, keeping the concentration of humic acid fixed (20 μg). In the first system, the 5.0 ml assay system contained 0.8 ml of 125 μg/ml humic acid (20 μg) and 0.1 ml, 0.25 ml, 0.5 ml, 1.0 ml and 2.0 ml of 500 μg/ml of HCH, corresponding to 10, 25, 50, 100 and 200 μg, respectively, and water to volume. In the second system in addition to 0.8 ml of 125 μg/ml humic acid, 0.5 ml of 0.5 μg/ml LAS corresponding to 0.05 ppm was present. To the third system, 0.8 ml of 125 μg/ml humic acid, 0.5 ml of 0.05 ppm LAS and 0.1 ml, 0.25 ml, 0.5 ml, 1.0 ml and 2.0 ml of 500 μg/ml HCH was added, corresponding to 10, 25, 50, 100 and 200 μg, respectively.

HCH-chlorophyll interaction

For this experiment 10 g loosely packed gamma-HCH coated sand (2 cm height) were taken in twelve 100 ml conical flasks. The experiments were performed in three sets. Each set consisted of four flasks. One each for 24, 48, 72 and 96 h. The first set comprised gamma-HCH coated sand with 20 fronds of *Lemna* in Hoagland's solution (30 ml). The second set used gamma-HCH coated sand with 20 fronds of *Lemna* in Hoagland's solution (30 ml) and LAS (5 μg/l) and the third served as controls containing Hoagland's solution, Lemna and sands without gamma-HCH. From each set 20 fronds of

Lemna were taken at 24, 48, 72 and 96 h and dried on filter paper, weighed and 1% homogenate was prepared in a Potter Elvehjem type homogenizer in 95% acetone. The homogenate was centrifuged at 2500 rpm for 10 min. Supernatants were separated and read at Spectronic 1001 spectrophotometer (bausch and Lomb, Milton Roy Company, USA) at 645 and 663 nm for chlorophyll present in lemna. The chlorophyll a and b concentrations were calculated by the formula of Arnon[17] in terms of mg/g fresh tissue weight. Percentage change in biomass of lemna was also calculated at above time intervals.

Fluorescence measurement

For chlorophyll, the fluorescence was read at excitation 600 nm and emission 662.4 nm and for humic acid, the fluorescence was measured at excitation 340 nm and emission 484.6 nm on a Shimadzu RF-5000, Ratio fluorophotometer (Japan).

Solubilization of gamma-HCH by detergents

Any effect of LAS on solubilization of gamma-HCH from sand was tested by shaking 2.0 g coated sand containing 1 mg gamma-HCH with 10.0 ml water or 10.0 ml 0.005 ppm LAS at different temperatures and estimating HCH in supernatant.

Estimation of HCH

The medium (water or LAS solution) containing gamma-HCH was decanted from each tube and extracted with 2 portions of 50 ml AR grade n-hexane in a separatory funnel. All the extracts were pooled, dried over anhydrous sodium sulphate, and filtered through glass wool. The filtered extract were evaporated and concentrated to 3.0–4.0 ml under control conditions to avoid loss of gamma-HCH by vaporization. Finally the volume was made up to 5.0 ml. The extracts were subjected to gas chromatographic analysis on a Varian Vista 6000. A 1.8 m x 4 mm column packed with chrome WHP mesh size 80/100 coated with 1.5% OV-17 and 1.95% OV-210 was used. Conditions (all temperature in °C): injector 250, column 180, detector 250; carrier gas nitrogen 60 ml/min. Electron capture detector ^{63}Ni was used.

RESULTS

The effect of gamma-HCH released from gamma-HCH coated sand on the fluorescence of humic acid in the presence and absence of LAS are depicted in Table 1. An increase in the fluorescence peak was noticed at 24 h (12%), 48 h (13.2%), 72 h (2.9%) and 96 h (22%) compared to the system containing sand alone (control) confirming the tendency of humic acid to bind with gamma-HCH. Presence of LAS led to an increase in the peak of fluorescence of humic acid 22% in 24 h, 18.9% in 48 h, 5.2% in 72 h and 28.5% in 96 h suggesting additive effect of LAS through solubilization of gamma-HCH. Further, LAS was found to solubilize gamma-HCH from 6 to 8 times at temperatures from 30 to 50°C compared to water. No further increase in gamma-HCH solubility can be seen above 50°C (Table 2).

Table 1 Effect of gamma-HCH and LAS on the fluorescence intensity of humic acid (arb. units).

Systems	*24 h*	*48 h*	*72 h*	*96 h*
Sand alone + Humic acid	184.2 ± 2.5	178.8 ± 2.1	174.9 ± 1.8	149.2 ± 1.6
Gamma-HCH loaded sand (500 μg/g) + Humic acid (50 mg/l)	207.8 ± 2.6	202.4 ± 2.4	179.9 ± 2.0	182.9 ± 2.3
Gamma-HCH coated sand (500 μg/g) + Humic acid (50 mg/l) + LAS (5 μg)	224.9 ± 3.2	212.7 ± 2.8	184.0 ± 2.2	192.2 ± 2.5

Values are represented as arithmetic mean of three replicates ± SE.

Table 2 Solubility of gamma-HCH in water and in LAS measured in terms of gamma-HCH released (ng/ml)

Temperature °C	*Solubility of gamma-HCH in water*	*Solubility of gamma-HCH in LAS (5 μg)*	*Difference in solubility*
30	0.049 ± 0.002	0.359 ± 0.017	0.310 ± 0.015
40	0.057 ± 0.003	0.325 ± 0.016	0.268 ± 0.013
50	0.060 ± 0.003	0.479 ± 0.02	0.419 ± 0.017
60	0.069 ± 0.004	0.347 ± 0.017	0.278 ± 0.013
70	0.068 ± 0.003	0.297 ± 0.014	0.229 ± 0.011

Values are represented as arithmetic mean of three replicates ± SE.

Variation in the concentration of gamma-HCH from 10 μg to 200 μg enhanced the fluorescence of humic acid from 9% to 51% as compared to fluorescence of humic acid (20 μg) when sand was not used. Addition of LAS to humic acid also enhanced the fluorescence of humic acid but the effect of gamma-HCH and LAS on the fluorescence of humic acid was almost similar to the effect of humic acid and gamma-HCH (Table 3).

Growth pattern of lemna did not show any significant change in biomass under any of the conditions up to 96 h and a marked change in the appearance of lemna was not noticed. Gamma-HCH exhibited a quenching effect on the fluorescence of chlorophyll at all the intervals but it was maximum at 24 h (14%) though quenching was not significant. The combined effect of gamma-HCH and LAS was found to enhance the fluorescence peak of chlorophyll at all the intervals. It was 20.5% at 24 h, 15% at 48 h, 14% at 72 h and 17% at 96 h as compared to the control. LAS alone was found to be even more effective in enhancing the fluorescence of chlorophyll in comparison to the combined effect of gamma-HCH and LAS. The increase in the fluorescence of chlorophyll was 25% in 24 h, 22% in 48 h, 26% in 72 h and 33% in 96 h in comparison to control (Table 4). From the data it is apparent that LAS, being a surface active agent, has got surface active potential to solubilize more of chlorophyll from the lemna thereby enhancing the fluorescence of chlorophyll.

A 38% increase in the fluorescence of humic acid was noticed in the system containing gamma-HCH coated sand after UVB irradiation (19 J) compared to the control (Table 5). Changes in other exposure times were not significant.

Table 3 Effect of various concentrations of gamma-HCH and LAS on the fluorescence intensity of humic acid (arb. units).

Systems	*Concentration of gamma-HCH (μg)*	*Concentration of LAS (μg/ml)*	*Fluorescence intensity of humic acid (arb. units)*
Humic acid (20 μg)	–	–	215.1 ± 10.2
Humic acid (20 μg) +	10	–	234.6 ± 11.2
Gamma-HCH	25	–	251.4 ± 12.5
	50	–	265.8 ± 12.6
	100	–	276.0 ± 13.2
	200	–	324.4 ± 15.4
Humic acid (20 μg) + LAS		0.05	243.5 ± 11.6
Humic acid (20 μg)	10	0.05	245.9 ± 11.8
Gamma-HCH + LAS	25	0.05	259.5 ± 12.4
	50	0.05	276.6 ± 13.2
	100	0.05	274.5 ± 13.1
	200	0.05	300.7 ± 14.6

Values are represented as arithmetic mean of three replicates ± SE.

Table 4 Effect of gamma-HCH and LAS on the fluorescence intensity of chlorophyll (arb. units).

Systems	*24 h*	*48 h*	*72 h*	*96 h*
Sand alone + Lemna	148.5 ± 2.2	153.6 ± 2.0	111.0 ± 1.6	103.6 ± 1.4
Gamma-HCH coated sand + Lemna	128.8 ± 1.8	143 ± 2.1	99.5 ± 1.3	94.4 ± 1.1
Gamma-HCH coated sand + Lemna + LAS	179.0 ± 2.4	176.8 ± 2.3	127.5 ± 1.9	121.6 ± 1.8
Sand + LAS + Lemna	185.2 ± 2.7	187.1 ± 2.5	140.0 ± 2.1	137.5 ± 2.0

Values are represented as arithmetic mean of three replicates ± SE.

Table 5 Effect of UVB irradiation on the fluorescence of humic acid in system with and without Gamma-HCH in the sand.

Dose of UVR irradiation in Joules (J)	*Exposure time in min*	*Fluorescence intensity of humic acid (arb. units)*	
		Sand + HA	*Gamma-HCH coated sand + HA*
3.8	30	160.8 ± 2.1	160.5 ± 2.0
7.6	60	164.3 ± 2.3	166.2 ± 2.2
11.4	90	166.3 ± 2.2	174.3 ± 3.0
15.2	120	163.4 ±2.2	179.4 ± 3.0
19.0	150	160.2 ± 2.0	221.6 ± 3.8
22.8	180	159.3 ± 1.9	163.9 ± 2.0
26.6	210	151.2 ± 1.85	161.2 ± 2.0
30.4	240	150.1 ± 1.82	163.2 ± 2.0

Values are represented as arithmetic mean of three replicates ± SE.

DISCUSSION

The physical state and environmental fate of inorganic and organic pollutants including metals, pesticides, PCB, PAH are complicated by the presence of humic and fulvic acid in the aquatic environment[18]. Humic substances in water can increase the apparent solubility of non-polar compounds through surfactant activity, binding inorganic and organic compounds either by covalent bonds, as charge transfer complexes, by hydrogen bonding or by Van der Waals interaction[18-20]. Factors like hydrophobic interactions may influence environmental persistence, intercompartmental distribution kinetics, bioavailability, biomagnification and target species and tissue effects[21,22].

The increase in the humic acid fluorescence peak after addition of gamma-HCH clearly indicated the binding between humic acid and gamma-HCH. Further increase in the fluorescence peak after addition of LAS could be due to its surface active action which helps in solubilization of gamma-HCH through partitioning. Also, the formation of semiquinone radical ions and a diamagnetic phenolate ion during interaction could be one of the contributing factors responsible for increase of fluorescence peak[23]. The quenching of fluorescence of chlorophyll after addition of gamma-HCH, is consistent with our earlir observation[24]. Again LAS was found to enhance the fluorescence of chlorophyll through solubilization of chlorophyll. The fluorescence quenching of chlorophyll in the case of *Lemna* treated with gamma-HCH could be due to electron withdrawing group present in gamma-HCH which makes free radical oxidation difficult.

The increase in the fluorescence of humic acid in the system containing sand coated with gamma-HCH after UVB irradiation indicated that humic acid act as a photosensitizer and may play a significant role in the detoxification of gamma-HCH in the presence of light and O_2 through HA-O_2 spin coupling.

CONCLUSIONS

From the above, it can be concluded that the gamma-HCH-humic interactions are of great significance in governing the fate and subsequently ecotoxicity of environmental chemicals in the aquatic ecosystem. Whether such interactions, depending upon the hydrophilicity of hydrophobicity of the compound, have any direct or indirect role in detoxification/potentiation of environmental chemicals in aquatic ecosystems needs further study.

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References

1. A. Bevenue, J. W. Hylind, Y. Kawano, and T. W. Kelly, *Pest. Monit. J.*, **6**, 57–63 (1972).
2. W. Salomons, N. M. de Rooij, H. Kerdijk, and J. Bril, *Hydrobiol.*, **149**, 13–30 (1987).
3. M. J. Lydy, and P. F. Landrum, *Aquat. Toxicol.*, **26**, 209–224 (1993).

4. P. S. Ziegenfuss, W. J. Renaudette, and W. J. Adams, in: *Aquatic Toxicology and Environmental Fate* (T. J. Poston, and R. Purdy, eds Vol. 9, STP 921 American Society for testing and materials Philadelphia, PA, 1986), pp 479–493.
5. H. Parlar, in: *Environmental fate of pesticides* (D. H. Hutson, and T. R. Roberts eds., Vol. 7, John Wiley & Sons Ltd., Chichester, 1990), p 245.
6. C. W. Carter, and T. W. Suffet, *Environ. Sci. Technol.*, **16**, 735–740 (1982).
7. S. A. Abdul, T. L. Gibson and D. N. Rai, *Environ. Sci. Technol.*, **24**, 328–333 (1990).
8. M. A. Gillott, G. L. Floyd, and D. V. Ward, *Environ. Entomol.*, **4**, 621–624 (1975).
9. W. Wang, and J. M. Williams, *Environ. Monit. Assess.*, **14**, 45–58 (1990).
10. H. Lal, V. Misra, P. N. Viswanathan, and C. R. Krishna Murti, *Ecotoxicol. Environ.Saf.*, **7**, 538–545 (1983).
11. G. Chawla, P. N. Viswanathan, and S. Devi, *Environ. Exp. Bot.*, **27**, 311–323 (1987).
12. V. Misra, V. Kumar, S. D. Pandey, and P. N. Viswanathan, *Water, Air, Soil Pollut.*, **50**, 233–239 (1990).
13. V. Misra, V. Kumar, S. D. Pandey, and P. N. Viswanathan, *Bull. Environ. Contam. Toxicol.*, **43**, 139–143 (1989).
14. V. Misra, V. Kumar, S. D. Pandey, and P. N. Viswanathan, *Arch. Environ. Contam. Toxicol..*, **23**, 54–58 (1992).
15. G. Chawla, in: *Manual on Aquatic Toxicology* (H. A. M. de Kruijf, D. de Zwart, P. N. Viswanathan, and P. K. Ray, eds. Allied Publishers, New Delhi, 1988), p. 298.
16. Environmental Protection Agency (EPA), Test methods for assessing the effects of chemicals in plants. EPA 560–17–75–008, final report, US Environmental Agency, Washington, DC (1975).
17. D. I. Arnon, *Plant Physiol.*, **24**, 1–16 (1949).
18. P. F. Landrum, S. R. Nihart, B. J. Eadla, and W. S. Gardner, *Environ. Sci. Technol.*, **18**, 187–192 (1984).
19. J. P. Hassett and M. A. Anderson, *Environ. Sci. Technol.*, **13**, 1526–1529 (1979).
20. G. E. Parris, *Environ. Sci. Technol.*, **14**, 1099–1105 (1980).
21. W. A. Bruggeman, in: *Handbook of Environmental Chemistry* (O. Hutzinger, ed. Vol. 2 Part B. Springer-verlag Berlin, 1982), pp 83–102.
22. A. T. Kan, and M. B. Tomson, *Environ, Toxicol, Chem.*, **9**, 253–263 (1990).
23. G. Tollin, T. Reid, and C. Steelink, *Biochim. Biophys. Acta.*, **66**, 444–447 (1963).
24. V. Misra, S. D. Pandey, and P. N. Viswanathan, *Bull. Environ. Contam. Toxicol.*, **56**, 809–816 (1996).