Bull. Environ. Contam. Toxicol. (1987) 38:500–508

Environmental Contamination and Toxicology

Influence of Suspended Solids on Bioavailability of Hexachlorobenzene and Lindane to the Deposit-Feeding Marine Bivalve, *Abra nitida* (Müller)

R. Ekelund,[1] Å. Granmo,[1] M. Berggren,[1] L. Renberg,[2] and C. Wahlberg[2]

The National Swedish Environment Protection Board, Kristineberg Marine Biological Station, Pl. 2130, S-450 34 Fiskebäckskil, Sweden

It is well-known that suspended solids in water adsorb hydrophobic pollutants (Hague et al 1974; Horzempa and di Toro 1983 a,b) thereby decreasing the bioavailability of the compounds to aquatic organisms which do not use the particles as food (Mc Leese and Metcalfe 1980; Eaton et al 1983). It is also known that hydrophobic pollutants deposited in sediments usually have a low bioavailability (Lee and Plumb 1974; Fulk et al 1975; Hirsch et al 1978). The relative contribution of water and sediment respectively to bioaccumulation of hydrophobic pollutants has been discussed in many papers based on field data (Courtney and Langston 1980; Murray et al 1981; Duinker et al 1983; Ray et al 1983), but the conclusions are uncertain. However, very few studies have been performed under controlled conditions in the laboratory on how the bioaccumulation of such compounds dissolved in the water is changed by adsorption to particles when these particles are used as food by the organism. This has now been investigated using the deposit-feeding bivalve Abra nitida which was exposed to hexachlorobenzene and lindane by means of a new method for dosing of hydrophobic compounds to a continuous flow system in long term tests.

MATERIALS AND METHODS

A continuous flow system was used (Fig.1) in which the flow of seawater was kept constant by means of a simple delivery box described by Granmo and Kollberg (1972). The suspended particles were introduced by a multichannel peristaltic pump (Ismatec SA) to the funnels inserted into the mixing cylinders and the stock suspension tank was equipped with a mechanical stirrer to keep the suspension homogeneous. The turbidity in 12 of the 24 tanks was kept at 30 NTU (nephelometric turbidity units, corresponding to 105 mg dry weight of particles/l for the sediment used), a value which is within the range of the natural variations along the

Present address: [1] The National Swedish Environment Protection Board. Kristineberg Marine Biological Station, Pl. 2130 S-450 34 Fiskebäckskil, Sweden, and
[2] The National Swedish Environment Protection Board. Special Analytical Laboratory, Box 1302, S-171 25 Solna, Sweden.

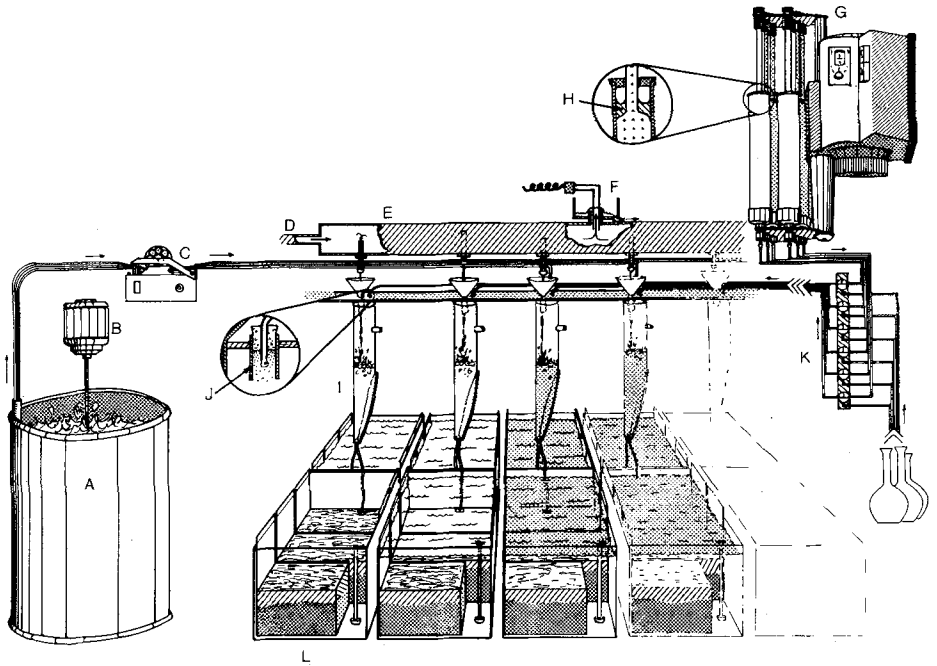

Figure 1. Continuous flow equipment for long term studies of bioaccumulation of highly hydrophobic pollutants in aquatic animals. A:stock suspension tank, B:stirrer, C:dosage pump, D:water inlet, E:water delivery system, F:level control, G:syringe pump, H:water-free glycerol, I:mixing cylinder, J:glass bell, K:three-way valves for refilling of test solution, L:test tanks.

coast. The water flow to the funnels was 150 ml/ min. and was subdivided by the cylinders to give a flow to each separate tank of 75 ml/ min. Temperature was held at +11 ±1 °C and salinity ranged between 31 and 33 °/₀₀. The turbidity/toxicant combinations used are shown in Table 1. Three separate tests (I, II and III) were

Table 1. Treatment combinations of hexachlorobenzene (HCB), lindane, acetone and the turbidity 30 NTU = 105 ppm particles in the turbidity tests with the deposit-feeding bivalve Abra nitida.

Treatment	Number of tanks
Control	2
Acetone	2
Particles	2
Particles + acetone	2
HCB + acetone	4
HCB + acetone + particles	4
Lindane + acetone	4
Lindane + acetone + particles	4

performed, each with a duration of 35 days. Precision infusion syringe pumps equipped with 50 ml special all-glass syringes (Braun Melsungen AG, Melsungen, W.-Germany) maintained the dosage rate of chlorinated hydrocarbons in acetone at 0.375 ml/h corresponding to an acetone concentration of 33 mg/l in the tanks. By turning the pumps 90 degrees, so that the syringes were in an upright position (Fig.1) and by filling the empty space above the plunger with water-free glycerol, which is immiscible with acetone, leakage between the walls of the cylinder and the plunger of the syringes was avoided. Polyethylene tubing was used throughout. The three-way valves, for renewal of stock solution (Fig.1) were made of Teflon. The end of the tube delivering the test solution was surrounded by a small glass bell to reduce evaporation of the acetone.

The soft bottom sediment was sieved through a 2 mm mesh screen. The sediment to be added in suspension was washed with fresh water to avoid aggregation of the particles and was decanted to obtain a defined particle size distribution. The sediment was characterized by a particle-counting device (Elzone 80XY, Particle Data Inc with software C.R. Hinze 80XY support system) with respect to number, size and approximate total surface area of particles entering and leaving the tanks; its organic content was also determined.

In order to estimate the degree of adsorption of the pesticides, water samples were taken from the tanks where particles had been added. The samples were then analysed for HCB and lindane before and after centrifugation at 24000 x g for 30 min.

Core samples (2.3 cm diameter) were taken to 1 cm depth in the sediment after 3 weeks of exposure, for determination of sediment-deposited pesticides.

Ten adult (10 ± 2 mm length) and 25 juvenile specimens (6 ± 1 mm) respectively of the detritus-feeding bivalve Abra nitida (Müller) were placed in each of the two sediment-filled boxes. Bioaccumulation studies were carried out on the adults while the juveniles were used to determine growth during the test. For this purpose all juveniles were marked at the edges of the shells. Animals which had not burrowed after 24 hours in the tanks were considered less viable and were replaced before commencing the experiment.

After three weeks of exposure, the box with adults was removed from each tank and the bivalves were kept in flowing sea water for 12 h to empty their guts of contaminated sediment. The shells were then removed and the soft tissues frozen. Each sample for analysis consisted of 40 specimens from four identical treatments; water and sediment samples were taken at the same time. Water samples were also taken regularly in all tanks. These samples (100 ml) were immediately extracted with n-hexane (5 ml), and the extracts were stored at 4 °C prior to analysis.

Finally, after 35 days, the juveniles were removed from the tanks and their shells were measured for determination of shell growth.

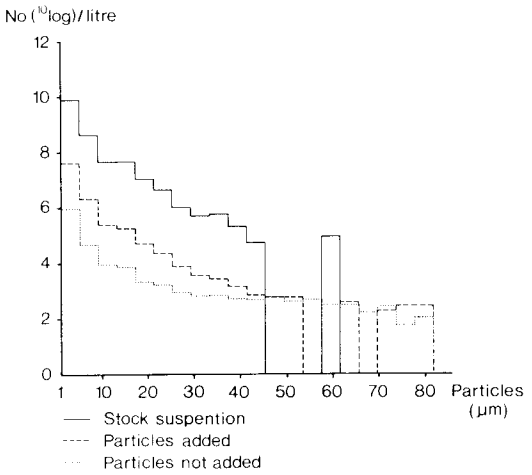

Figure 2. Size distribution of particles in water sampled from the aquaria and from the stock suspension in a bioaccumulation/turbidity test involving Abra nitida and the pesticides hexachlorobenzene and lindane.

All chemicals used in the analyses were tested in blank procedures by gas chromatography. Aldrine was used as an internal standard for the gas chromatographic quantification.The soft parts of the organisms were extracted according to the method of Jensen et al (1979). However, the evaporation step was replaced by a process involving drying the extracts with magnesium sulphate and adjusting the volume to 5.0 ml with a nitrogen stream and a water bath. Prior to the gas chromatographic determination an aliquot of each extract (2 ml) was treated with an equal volume of sulphuric acid monohydrate, to remove the lipids.

For the lipid determination identical samples were extracted as previously described. After evaporation of solvent the lipid content was determined gravimetrically.

The sediment samples from the tanks were extracted for HCB and coextractives were removed according to Jensen et al. (1977). For the quantification of lindane and HCB a Varian 6000 gas chromatograph equipped with a 63Ni electron capture detector was used together with a Varian Vista 401 system for processing of the chromatographic data. The chromatographic column was a 25 m fused silica BP-5 (SGE) with an inner diameter of 0.25 mm. Helium was used as carrier gas (1.20 ml/min) and nitrogen as make-up gas (30 ml/min).

The sample extracts were diluted with hexane containing an internal standard. They were injected together at 80 °C. After one minute, the temperature was raised 30 °C/min to 220 °C and then 8 °C /min to 260 °C. The injector and detector temperatures were held at 220 °C and 320 °C, respectively.

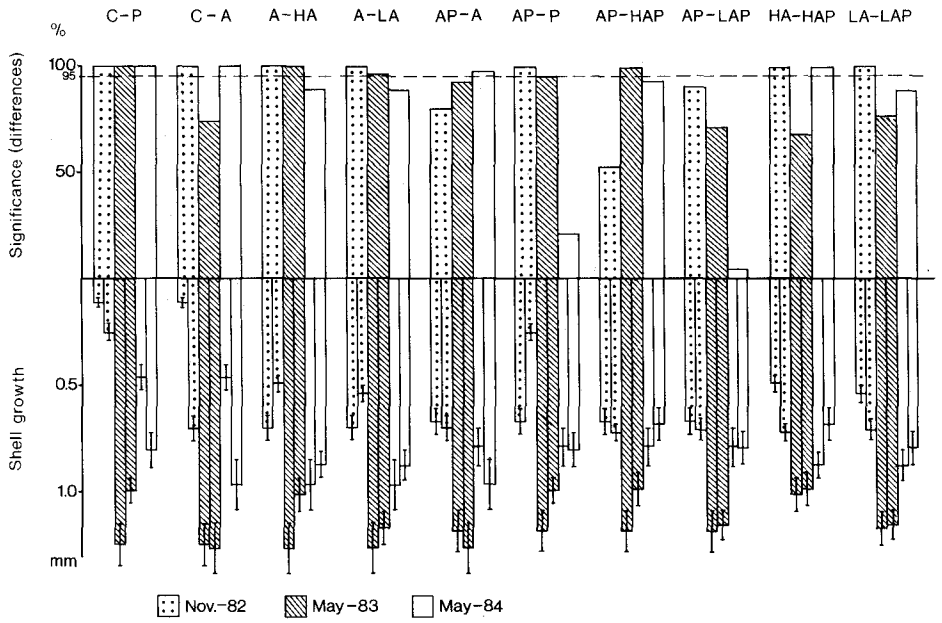

Figure 3. Shell growth (mm) ± 95% confidence limits of Abra nitida exposed to hexachlorobenzene and lindane for 35 days at low and high turbidity. In the experiments from Nov.-82 and May-83 the values from all treatments did not fit a normal distribution, so a nonparametric test was chosen (Wilcoxon rank test for two groups). The values from May -84 were all normally distributed and hence a parametric test was chosen (one-tailed ANOVA). Significance of difference between treatments is also indicated by columns. C= controls, P= added particles (high turbidity: 30 NTU= 105 ppm), A= acetone, H= HCB, L= Lindane.

RESULTS AND DISCUSSION

The results of the particle counts are shown in Fig.2. From a comparison of the size distribution of particles in water entering and leaving the turbid aquaria it was calculated that the particles settling in the tanks corresponded to about 30% of the total surface area of the added particles. The organic content of the particles was about 5 %.

Both pesticides slowed growth a little, but suspended particles did not seem to affect this inhibition (Fig. 3). The animals sampled during spring had a higher content of lipids (1.5%) than in the autumn samples (0.9%).

The total concentration of organochlorine in water varied between the different tests, especially for HCB. The mean concentrations in the three tests were estimated and are given in Table 2. For a fair judgement of the results it is most relevant to compare the accumulation ratios, that is, the quotient between the concentration in animals or sediment and the total mean concentration in the water. The results are presented in Fig. 4 and they show that

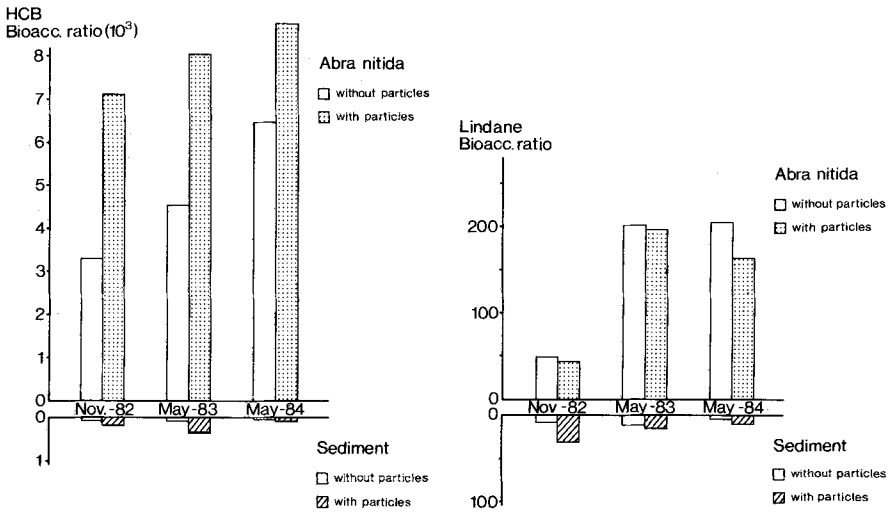

Figure 4. Accumulation ratios (quotient between the concentration in animals or in sediment and the total mean concentration in water) of hexachlorobenzene and lindane in the detritus-feeding bivalve Abra nitida and in the sediment exposed for 19 days at low and high turbidity.

Table 2. Total mean concentrations (dissolved + particle-bound), ppb, of hexachlorobenzene (HCB) and lindane in water samples from three turbidity tests with Abra nitida.

Test	Treatment			
No	HCB	HCB+particles	Lindane	Lindane+particles
I	3.1	1.3	7.5	6.7
II	3.8	4.0	8.5	8.2
III	6.5	7.8	8.8	8.0

the sediment became more contaminated in the presence of suspended solids. Also the accumulation ratio of Abra was higher in all the three experiments performed, when suspended particles were added. For the less hydrophobic lindane the corresponding values were much lower and the bioaccumulation in the presence or absence of suspended particles was about the same. Centrifugation of the water samples showed that the major part of HCB in water (>95%) was particle-bound at high turbidity whereas only about 12% of lindane had been adsorbed onto particles: this accords with the higher levels of HCB, compared with lindane in the sediment.

For lindane the steady state level was probably achieved; following this the amount of compound accumulated became related to lipid content of the animals; this is reflected in the lower bioaccumulation ratio of Lindane in the autumn test (Fig. 4).

The difficulties of dosing hydrophobic compounds in a continuous flow system are well known. None of the existing methods was considered to have enough safety and accuracy for dosing in long term experiments and therefore we were forced to develop a new technique. In our experiments we used acetone as a solvent, which has a good capacity to dissolve highly hydrophobic compounds, is completely miscible with water and has a low toxicity to aquatic organisms (Johnson and Finley 1980). However, the problems connected with the volatility of acetone and its corrosive action upon tubes, plastics etc had to be solved. It was necessary to use dosage pumps with a very low pumping rate, to minimize the amount of acetone in the tanks.

The results from all the tests show that the accumulation ratio of HCB for the animals became much higher in the concomitant presence of added particles, whereas in the case of the less hydrophobic lindane the corresponding ratio seemed to be unaffected by suspended solids. Even if it is not known whether our results represent the steady state, the HCB level in A. nitida would probably be highest at the higher turbidity also at steady state. This assumption may be supported by the results from Landrum and Scavia (1983) who, by extrapolation in time, calculated that the detritus-feeding amphipod Hyalella azteca accumulated more anthracene in the presence of fine-grained sediment which bound part of the compound.

It is to be expected that adsorption of a hydrophobic compound to particles will cause a decrease of its availability to aquatic organisms which do not utilize the particles as food, as equilibrium partitioning against the surrounding water, via the gills and skin, then has a determining importance for the steady state level of the pollutant in the animal. However, the consequence of particle adsorption of a hydrophobic pollutant for its bioavailability to filter feeders and deposit feeders ingesting the particles is not so easy to predict. For these animals this phenomenon may either cause a decreased bioavailability, if equilibrium partitioning via the gills is still the decisive factor for bioaccumulation, or possibly an increased bioavailability, if uptake by food determines the steady state concentration of the compound in the organism. The relative importance of the two ways of uptake and the occurrence of a true biomagnification is still a matter of conjecture (Hamelink et al 1971; Goerke et al 1979; Ernst 1980; Bruggemann 1982; Schneider 1982; Borgmann and Whittle 1983; Moriarty 1983) and may vary greatly depending on the morphology, physiology and the feeding habit of the organism.

When considering animals living in the sediment one should be aware that the contact of the outer body surfaces with a contaminated sediment, which is in equilibrium with the pollutant in the water column, does not infer a heavier exposure compared to contact with the water itself. That is, it is not the higher concentration, as such, but the fugacity (which is related to the chemical potential or Gibbs free energy) of the compound in the sediment which determines the bioavailability. This has the same value

on the particles and in the water if the two compartments are in equilibrium with each other. Furthermore, when the uptake of a hydrophobic pollutant, via the gut, is considered, one should keep in mind that the major part of the food of deposit feeders consists of indigestable material, which has the potential to adsorb pollutants liberated from the food particles being digested. The increased bioaccumulation of HCB in Abra at the higher turbidity in spite of a greatly lowered concentration of dissolved compound, gives a level in the animals which is much higher than can be explained by only passive equilibrium partitioning via the gills and skin. Instead the results indicate that, in this case, the uptake via food predominates strongly over the former exchange process.

Acknowledgements. Thanks are due to the Royal Swedish Academy of Sciences and the director of Kristineberg Marine Biological station, professor J.O. Strömberg for providing laboratory facilities. Thanks are also due to Dr A. Josefsson for fruitful discussions. Financial support was given by the National Swedish Environment Protection Board (Contract No. 5333025-4).

REFERENCES

Borgmann U, Whittle DM (1983) Particle-size-conversion efficiency and contaminant concentrations in Lake Ontario biota. Can J Fish Aquat Sci 40:328-336

Bruggemann WA, (1982) Hydrophobic interactions in the aquatic environment. In: Hutzinger BO (ed) The handbook of environmental chemistry, Vol 2. Springer -Verlag. Berlin, Heidelberg.

Courtney WAM, Langston WJ (1980) Accumulation of polychlorinated biphenyls in turbot (Scophtalmus maximus) from seawater sediments and food. Helgoländer Meeresunters 33:333-339

Duinker JC, Hillebrand MTJ, Boon JP (1983) Organochlorines in benthic invertebrates and sediments from the Dutch Wadden Sea; Identification of individual PCB-components. Neth J Sea Res 17:19-38

Eaton JG, Mattson VR, Mueller LH, Tanner DK (1983) Effect of suspended clay on bioconcentration of Kelthane (trade name) in fathead minnows. EPA-600/3.-83-0.69 NTIS order No PB 84-109990.

Elder DL, Fowler SW, Polikarpov GG (1979) Remobilization of sediment-associated PCBs by the worm Nereis diversicolor. Bull Environ Contam Toxicol 21:448-452

Ernst W (1980) Effects of pesticides and related organic compounds in the sea. Helgoländer Meeresunters 33:301-312

Fulk R, Gruber D, Wulschleger R (1975) Laboratory study of the release of pesticide and PCP materials to the water column during dredging and disposal operations. US Army Corps of Engineers. Contract Report D-75-6 prepared by Envirex, Inc Milwaukee, Wisconsin.

Goerke H, Eder D, Weber K, Ernst W (1979) Patterns of organochlorine residues in animals of different trophic levels from the Weser estuary. Mar Poll Bull 10: 127-133

Granmo Å, Kollberg SO (1972) A new simple water flow system for accurate continuous flow tests. Water Res 6:1597-1599

Hague R, Schmedding DW, Freed VH (1974) Aqueous solubility, adsorption, and vapor behaviour of polychlorinated biphenyl Arochlor 1254. Environ Sci Technol 8: 139-142
Hamelink IL, Waybrant RC, Ball RC (1971) A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in benthic en-vironments. Trans Amer Fish Soc 100:207-214
Hirsch DH, Di Salvo LH, Peddicord R (1978) Effects of dredging and disposal on aquatic organisms. U.S. Army Corps of Engineers, Technical Report DS-78-5 pre-pared by the Naval Biosciences Laboratory, University of California, Naval Supply Center, Oakland, California.
Horzempa LM, Di Toro DM (1983a) The extent of reversibility of polychlorinated biphenyl adsorption. Water Res 17:851-860
Horzempa LM, Di Toro DM (1983b) Polychlorinated biphenyl partitioning in sediment-water systems: The effect of sediment concentration. J Environ Qual 12: 373-380
Jensen S, Renberg L, Reutergårdh L (1977) Residue analysis of sediment and Sewage Sludge for organochlorines in the presence of elemental sulfur. Anal Chem 49: 316-318
Jensen S, Reutergårdh L, Jansson B (1979) Seventh FAO/SIDA workshop on aquatic pollution in relation to protection of living resources. Manila, The Phillipines. May 7-June 9, 1979. Workshop Manual:part 3
Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. US Dep Int Fish Wildl Serv Resour Publ 137, Washington, DC. 98 pp
Landrum PF and Scavia D (1983) Influence of sediment on an anthracene uptake, depuration and biotransformation by the amphipod Hyalella azteca. Can J Fish Aquat Sci 40:298-305
Lee GF, Plumb RH (1974) Literature review of research study for the development of dredged material disposal criteria. U.S. Army Corps of Engineers. Contract report D-74-1 prepared by the Institute for Environmental Studies, University of Texas, Dallas
Moriarty F (1983) Ecotoxicology. The study of pollutants in ecosystems. Academic Press. London.
McLeese DW, Metcalfe CD (1980) Toxicities of eight organochlorine compounds in sediment and seawater to Crangon septemspinosa. Bull Environ Contam Toxicol 25:921-928
Murray HE, Ray LE, Giam CS (1981) Analysis of marine sediment, water and biota for selected organic pollutants. Chemosphere 10:1327-1334
Ray LE, Murray HE, Giam CS (1983) Analysis of water and sediment from the Nueces estuary/Corpus Christi Bay (Texas) for selected organic pollutants. Chemosphere 12:1039-1045
Schneider R (1982) Polychlorinated biphenyls (PCBs) in cod tissues from the Western Baltic: Significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gillbreathing animals. Meeresforschung 29:69-79

Received April 4, 1986; accepted July 29, 1986.