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Do Carrier Solvents Enhance the Water Solubility of Hydrophobic Compounds?

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Aquatic toxicity testing of potentially toxic substances is now accepted as an important aspect of environmental hazard evaluation. In conducting aquatic toxicity tests, ideally, the test organism is exposed to the chemical dissolved in water. To achieve homogeneous distribution of the hydrophobic test chemical in water is a problem owing to the poor solubility of these substances, especially those that are of maximum interest in hazard evaluation because of their persistent nature and tendency for bioaccumulation. This problem is met more often in conducting acute or short-term toxicity tests, where amounts higher than the water solubility limits of hydrophobic compounds are reported to have been employed sometimes. For instance, against a known water solubility of DDT of less than 2 ug/L, the reported 96-h LC50 values for several species of aquatic organisms range from a few to a few hundred ug/L (see for instance Table 1 in US EPA Ambient Water Quality Criteria for DDT). Traditionally this problem has been sought to be overcome by using a carrier solvent, as the hydrophobic compounds are moderately to highly soluble in a few water-miscible organic solvents. In a recent review on aquatic toxicity testing, Buikema et al. (1982) stated "Toxicity tests conducted with water insoluble chemicals, e.g., many biocides, usually use a solvent to enhance solubility". Fujiwara (1979) stated that according to the 'Chemical Substances Control Law of Japan' a suitable solubiliser may be used in conducting the 48-h toxicity test, if the chemical is poorly soluble in water. In the OECD guidelines too (OECD Guidelines 1981) the use of suitable solubilisers is recommended in conducting aquatic toxicity tests with poorly water-soluble compounds. In all these cases, it is hoped that the use of a carrier solvent somehow enhances the water solubility of such poorly water-soluble compounds, thereby

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enabling the use of amounts higher than the limit of saturation of that chemical in water. To our knowledge, there have been no published reports stating that a carrier solvent, in the range of concentrations recommended for use in aquatic toxicity tests, increases the water solubility of organic compounds. Hence we studied the water solubility of three compounds viz., dieldrin, nitrofen and captan, in the presence or absence of three concentrations of acetone.

MATERIALS AND METHODS

The water solubility of the three pesticides with or without acetone was studied using a sand column similar to that described by Chadwick and Kiigemagi (1968). Merck sea sand (no. 7710) was heated for two hours at 600°C, cooled, washed with deionized water and dried at 110°C. The dried sand was packed on a chromatographic column (180 x 12 mm), fitted with a fritted disc and stopcock. Dieldrin (99.5% pure, 30 mg) or nitrofen (99.5% pure, 100 mg) was dissolved in 2 mL acetone and added to the column. The solvent was made to run down the column by the application of gentle vacuum and the column was allowed to dry. For captan, this procedure did not result in uniform deposition and elution of the compound. Hence 120 mg of captan (97.6% pure) was dissolved in 20 mL of 1:1 ethyl acetone and toluene and was deposited on 50 g washed sand in a 50 mL round-bottomed flask. After removing the solvent on a rotary flash evaporator, the sand was packed on a 30 cm-long chromatographic column. With all the three compounds, the column was eluted first with 750 mL deionized water and then successively thrice with 400 mL water containing acetone at a concentration of 10, 100 and 500 uL/L, at an elution rate of 2.5 mL/min. The first 500 mL water eluate was discarded and five samples were collected into 50 mL volumetric flasks. Similarly, with each concentration of acetone, the first 150 mL eluate was discarded and five samples were collected into 50 mL volumetric flasks. The compound was extracted from the 50 mL sample in the volumetric flask by shaking with 2 mL toluene (hexane in the case of dieldrin), which after appropriate dilution was injected into a gas chromatograph (Varian model 1740 with a ^{63}Ni electron capture detector). Three different 2 m-long columns were used for the three compounds viz., 1.2% Dexsil 300 GC on 80/100 mesh Chromosorb 750 at 225°C for dieldrin, 8% DC 550 + 2% DC 200 on 80/100 mesh Gaschrom Q at 240°C for nitrofen and 3% OV 101 on 80/100 mesh Chromosorb W-DMCS at 200°C for captan. Other operating conditions were the same for all the three compounds and were-injector 190°C and detector 290°C.

Fortification of water and subsequent extraction and analysis were carried out in triplicate with each com-

pound to calculate the percentage recovery. The water solubility of the three compounds under different conditions of treatment was tested for significant difference by analysis of variance.

RESULTS AND DISCUSSION

The percentage recovery of dieldrin, nitrofen and captan from fortified samples was 94.7 ± 3.4%, 101.8 ± 3.4% and 94.1 ± 0.85% respectively. The results of the study on the water solubility of the three compounds are shown in Table 1. Analysis of variance showed no significant difference in the solubility of dieldrin or nitrofen in water in the presence or absence of acetone at any of the three concentrations ($F = 2.04$ and 2.34 respectively; not significant at $p = 0.05$ and at 3 and 16 degrees of freedom). For captan, there was a significant difference in its water solubility among different treatments ($F = 43.69$, significant at $p = 0.005$ and at 3 and 16 df).

Table 1. Solubility (ug/L)§ of dieldrin, nitrofen and captan in water with or without acetone.

Compound	without acetone	with 10 uL/L	with 100 uL/L	with 500 uL/L
Dieldrin	250 ± 4	252 ± 3	252 ± 6	256 ± 2
Nitrofen	609 ± 22	593 ± 19	594 ± 35	565 ± 27
Captan	4642 ± 144	5328 ± 274	5992 ±337	6526 ± 319

§ All values are corrected to the nearest whole number; each value is a mean of five determinations with the standard deviation indicated.

The water solubility of dieldrin or nitrofen was not increased by the addition of acetone and there was only a 41% increase in the solubility of captan in the presence of the highest concentration of acetone usually employed in aquatic toxicity tests. The degree of influence of acetone on the water solubility of hydrophobic compounds is perhaps related to the extent of the water solubility of the compound itself - water solubility of poorly soluble compounds not being influenced by acetone at all, and those of slightly more soluble compounds being increased a little in the presence of acetone. The extent of this influence, however, seems to be limited, because in a study on the water solubility of atrazine, Fürer and Geiger (1977) observed only a 16.6 to 33.2% increase in its water solubility in the presence of 1% acetone. Such a small increase in the water solubility is of little practical significance in conducting acute aquatic toxicity

tests except in rare instances where the slope of the dose-mortality regression line is very steep.

The solubility of dieldrin and nitrofen in water, calculated by us, is in agreement with the values reported in the literature. For captan, however, our value is about nine times that reported in the Pesticide Manual (Martin and Worthing 1974), which is often cited as the source of this information by other workers. Our value for captan, likewise, differs from that of Fürer and Geiger (1977). This discrepancy may be due to the different methods adopted to calculate the water solubility. Todd et al. (1980) reported that they obtained widely differing values for the same compound when they adopted different methods to calculate its water solubility. In the Pesticide Manual, the method adopted for determining the water solubility of captan is not mentioned; Fürer and Geiger (1977) followed a stepwise dilution of a fine suspension of the compound in water and determined the point of sudden decrease in turbidity. As has been pointed out by Gunther et al. (1966) the solubility data reported earlier for many compounds need to be reevaluated.

Acetone is one of the most often used carrier solvents in conducting aquatic toxicity tests, along with dimethyl formamide and ethanol. For instance, in 1587 acute toxicity tests with 271 chemicals and 58 test species, conducted over a period of 14 years at the Columbia National Fisheries Research Laboratory, in USA, acetone -at a concentration not exceeding 0.5 mL/L- was often used as a carrier solvent in preparing the test solutions (Johnson and Finley 1980). In the light of our study, the usefulness of acetone in increasing the water solubility of hydrophobic compounds seems negligible. Since it is easier to prepare the test medium while employing a carrier solvent, and since earlier studies by one of us (Murty and Hansen 1983, Murty in preparation) have shown no interaction between the carrier solvent (acetone or ethyleneglycol monoethyl ether) and the toxicant, and that the carrier solvent does not alter the extent of uptake of the toxicant by the test organism, the use of acetone as a carrier solvent may be continued only for the purpose of easier preparation of the test medium and not for increasing the water solubility of poorly water -soluble compounds.

If the use of a carrier solvent does not help increase the water solubility of hydrophobic compounds, what is the validity of all the LC50 values reported in the literature that exceed the saturation value of that compound in water? Since neither the use of a carrier solvent nor any of the devices designed to generate saturated water solutions of test chemicals (Chadwick and Kiigemagi 1968, Veith and Comstock 1975) can put more chemical into water than its water solubility limit, all those toxicity tests conducted with an amount of the

substance in water higher than its saturation value in water seem to be meaningless. Although that much chemical may have been added to the test medium, the real concentration of the test chemical in the test tanks cannot exceed its solubility in water, the rest being adsorbed to walls, being present in suspension or precipitation or lost through evaporation. Hylin (1980) has emphasized that that part of the chemical that is not in true solution is not really biologically active and available. Further, Herzel et al. (1976) have shown that in aquatic toxicity tests conducted at a concentration equal to, or near the saturation of the test chemical in water, the evaporation loss was very high. Thus it is obvious that in all such cases where the calculated LC50 value exceeds the water solubility of a compound, the test organism is not exposed to those amounts of the toxicant supposed to be present in the test tanks, but to a much lesser and unknown concentration. The scientific indefensibility of such data in the light of our present findings will be discussed elsewhere (Murty and Herzel, in preparation). It must also be emphasized that when other carrier solvents like dimethyl formamide and ethanol are also found ineffectual in increasing the water solubility of hydrophobic compounds, thus confirming our observations with acetone, the 'Toxicity Testing Protocols', 'Guidelines' and the like that recommend the use of a carrier solvent for enhancing the solubility of poorly water-soluble compounds need to be altered. Similarly, the relevant portions of the environmental protection laws in force in various countries, that insist on the submission of pre-registration data on acute toxicity to a few representative aquatic organisms, irrespective of the water solubility of such compounds (even when the reported LC50 values exceed the water solubility of that compound), have to be suitably altered in the light of this new knowledge.

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