

PROBLEMS ASSOCIATED WITH LOW-SOLUBILITY COMPOUNDS IN AQUATIC TOXICITY TESTS: THEORETICAL MODEL AND SOLUBILITY CHARACTERISTICS OF AROCLOR® 1254 IN WATER*

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Abstract—A theoretical model of the behavior of substances having low water-solubility is presented and discussed with respect to aqueous bioassay. Ultracentrifugal techniques were used in an attempt to study size distributions of Aroclor 1254 aggregates in aqueous emulsions. Results indicate strong adsorption from emulsion by surfaces and a water-solubility at 20°C of less than $0.1 \mu\text{g l}^{-1}$ in distilled water and approximately 40% of that value in water containing 30 g l^{-1} NaCl. Implications with regard to aqueous bioassay are discussed.

INTRODUCTION

Laboratory experiments designed to determine the effects of chemicals on aquatic organisms require that the tests be conducted under conditions which reproduce those present in nature as closely as possible. In order to accomplish this in a precise and scientific fashion, the physical state of a compound in an aqueous dispersion must be known. Convenience, time and other factors have in the past often led to the use of techniques in the laboratory which do not take into consideration that the solubility characteristics of a compound may possibly affect the toxicity necessitating extrapolation from an apparent toxicity established in the laboratory to an expected toxicity under field conditions. In many instances, the practice of using extrapolation in scientific investigations is necessary and has proven to be a valuable tool when certain conditions cannot be met. However, the range through which the extrapolation is carried out must be chosen with great care, because without sufficient experimental and theoretical justification, a resulting extrapolation in this light may well prove to be unrealistic. Since natural water conditions represent a multi-component system, any attempt to understand it quantitatively must be preceded by a study of the system under ideal conditions. While the knowledge thus gained may or may not be of consequence in direct application, it, nevertheless, provides a more precise scientific basis for choosing valid limits for extrapolation.

The physical state of a compound in water is not a simple and straightforward phenomenon, even given

the idealized conditions of a two-component system—a single solute and a single solvent. A definable system should, however, be the starting point of any scientific investigation aimed to arrive at data which lead to a quantitative understanding of the behavior of a compound in water. With these data a more precise attempt can be made to extrapolate from a system employed in the laboratory to the obviously much more complex system present in natural waters.

The purpose of this work is to provide a working theory on the behavior of substances of low water-solubility and to test this theory by investigating the solubility characteristics of Aroclor 1254.

THEORY

To explain and predict the characteristics of water-insoluble substances at low concentrations, an attempt is made here to redefine the basic principles underlying a disperse system. No attempts have been made to include in the definition the somewhat obsolete and often vague definitions of emulsions, suspension, colloids, etc. The characteristics ascribed to each becoming readily apparent as the theoretical treatment of the proposed model continues.

In this paper, an ideal or true solution is defined as a solute dispersed in a solvent so that any single molecule of solute is surrounded by enough solvent molecules to ensure that at any instant all solute molecules are distributed statistically equidistant, assuming a dilution at which interactions between solute molecules become negligible.

The ideal solution, under the conditions described, is represented by the presence of single solute molecules. Solute aggregates consisting of two or more molecules may represent a deviation from the ideal solution because, at least theoretically, these aggregates could consist of any number of molecules whose behavior would not necessarily coincide with that of

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a single molecule. For each solute and a single solvent, there is assumed to exist amongst all aggregates a maximally stable aggregate which, due to its nature, remains statistically equidistant from all other aggregates for at least a certain period of time. The stability of this aggregate depends solely on the molecularly characterized interactions at the solute-solvent interphase and on temperature.

By definition, a single solute molecule in a disperse system possesses a certain sphere of influence, the nature of which governs the fate of the solvent molecules that surround it, which in turn affects the behavior of the solute molecule, and thus determines the characteristics of the solute molecule in the system. While precise information is lacking, it is known, nevertheless, that the range of effect of a solute molecule may extend through several layers of surrounding solvent molecules. This means, of course, an orderly alignment involving either oppositely charged polar regions or non-polar regions on the solute and the solvent molecules. If this interaction between solute and solvent molecules is of significance, the above defined ideal solution can be visualized, provided also that there is no competition among the solvent molecules belonging to respective spheres of influence of two separate solute molecules.

The complexity of the situation is increased in cases where the interactions between solute and solvent molecules (solute-solvent interactions) become less pronounced, and, as a result, the interactions between solute and solute molecules (solute-solute interactions) become more pronounced. This implies that the sphere of influence around the solute molecule is diminished with respect to the solvent molecules which are now no longer attracted to the same degree. As two or more solute molecules start to form aggregates, the factor of size of aggregates versus their stability in a solvent becomes of utmost importance.

A generalized illustration of the size distribution of aggregates that one might expect to find in a suspension is shown in Fig. 1. Region "A" describes an area in which the aggregates are too small to exist independently because interactions in the sphere of influence at that point are such that solute-solute interactions, which have now become aggregate-aggregate interactions, are more pronounced than the aggregate-solvent interactions. Therefore, these aggregates are expected to coalesce, moving them into region "B", which describes a range of aggregate sizes of maximum stability. The aggregate-aggregate interactions in this range are weaker than in region "A" for that size of aggregate. Region "C" described aggregates which are too heavy to remain in suspension for a given period of time and will settle out or break into smaller, more stable aggregates. The exact shape of this curve and especially that of region "B", depends on how tightly the solvent is held within the

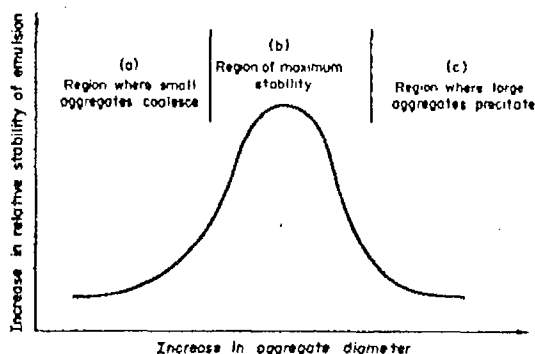


Fig. 1. Theoretical relative stability of different sizes of aggregates in an emulsion during a given time interval.

sphere of influence of the solute aggregate, which is a function of the molecular interactions between solute and solvent.

The distribution of different aggregate sizes in terms of molecularly characterized interactions is shown in Fig. 2. The actual equilibrium reaction taking place is described in a simplified manner at the top of the figure. The two curves relate the hypothetical strength of interactions of solute-solvent (aggregate-solvent) type and solute-solute (aggregate-aggregate) type to aggregate size. The region where the curves cross corresponds to a distribution of aggregate sizes of maximum stability.

MODEL

Aroclor 1254 was chosen as a model compound because it has been extensively used in bioassay at this laboratory (Duke, Lowe and Wilson, 1970; Nimmo *et al.*, 1971a; Nimmo *et al.*, 1971b; Hansen *et al.*, 1971; Lowe *et al.*, 1972; Walsh, 1972; Cooley, Keltner and Forester, 1972).

One approach to estimate quantitatively the solubility of Aroclor 1254 in water and the behavior of its aggregates is to use ultracentrifugal analysis. This technique permits the selective removal of particles of a certain size. For a spherical particle having a density of (ρ) and a radius of (r) the molecular weight (mol. wt) is represented by:

$$\text{mol. wt} = 4/3\pi r^3 \rho N_0 \quad (1)$$

where N_0 is Avogadro's Number.*

Two opposing forces (f) which determine the fate of a

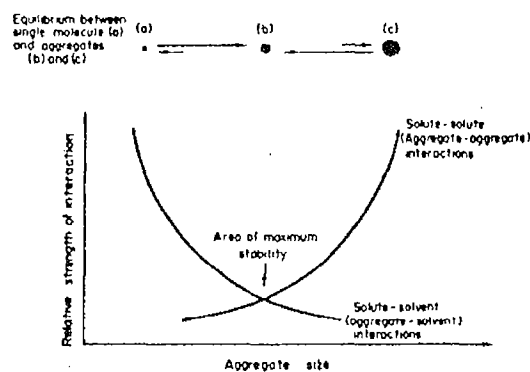


Fig. 2. Theoretical strength of interaction between solute and solvent.

* The equations used are normally found in any textbook on physical chemistry, and their reproduction here is intended merely for the convenience of the reader.

particle in solution:

$$\text{sedimentation } f = 4/3\pi r^3(\rho - \rho_0)g \quad (2)$$

and

$$\text{bouyancy } f = 6\pi r\eta, \quad (3)$$

where (ρ_0) is the density of the solvent, (g) is gravity, and (η) is the viscosity of the solvent.

To remove a small particle from an emulsion at a reasonable rate, a force larger than gravity must be applied. Using the ultracentrifuge, (g) in equation (2) is replaced with ($\omega^2 x$), the angular velocity of the centrifuge rotor (ω) times the distance of travel (x) of the emulsified particle.

The rate of sedimentation during centrifugation is described by:

$$\frac{dx}{dt} = \frac{2r^2(\rho - \rho_0)\omega^2 x}{9\eta} \quad (4)$$

where (t) is time in seconds to reach equilibrium. Integration yields:

$$\ln x_2 - \ln x_1 = \frac{2r^2(\rho - \rho_0)\omega^2 t}{9\eta} \quad (5)$$

The radius of a spherical particle is then given by:

$$r = \left[\frac{9\eta(\ln x_2 - \ln x_1)}{2(\rho - \rho_0)\omega^2 t} \right]^{1/2} \quad (6)$$

Where

$$\omega = 0.10472 \text{ (rev min}^{-1}\text{) rotor}$$

$$\eta = \text{g cm}^{-1} \text{ s}^{-1}$$

$$\rho = \text{g cm}^{-3}$$

$$x = \text{cm}$$

$$t = \text{s}$$

Knowing the radius of a particle or assuming a radius, the time necessary to remove the particle from an emulsion is given by:

$$t = \frac{9\eta(\ln x_2 - \ln x_1)}{2(\rho - \rho_0)r^2\omega^2} \quad (7)$$

The following are particle size limits calculated using equation (6) for given centrifugation times, with $\eta = 8.94 \times 10^{-3} \text{ g s}^{-1} \text{ cm}^{-1}$, $x_1 = 6.7 \text{ cm}$, $x_2 = 15.3 \text{ cm}$, $\rho - \rho_0 = 0.508 \text{ g cm}^{-3}$ at 25,000 rev min⁻¹.

Time (h)	Radius of particle (nm)
1	16.3
2	11.5
3	9.3
4	8.1
6	6.6
8	5.7

The following are particle size limits calculated using equation (6) for given centrifugation times, with $\eta = 8.94 \times 10^{-3} \text{ g s}^{-1} \text{ cm}^{-1}$, $x_1 = 6.00 \text{ cm}$, $x_2 = 10.73 \text{ cm}$, $\rho - \rho_0 = 0.508 \text{ g cm}^{-3}$ at 45,000 rev min⁻¹.

Time (h)	Radius of particle (nm)
1	7.6
2	5.4
3	4.4
4	3.8 (208,000 g mole ⁻¹ *) 636 molecules)
6	3.1
8	2.7
12	2.2 (40,000 g mole ⁻¹ *) 124 molecules)

* Average mol. wt Aroclor 1254 = 327 g mole⁻¹ (Hutzing, Safe and Zitko, 1972).

EXPERIMENTS WITH AROCLOR 1254

Wide-mouth jars, 30 cm high and 14 cm wide, were used to produce 3 l. of Aroclor 1254 emulsion per batch. Mechanical considerations concerning the proper physical agitation of Aroclor 1254 and water made it necessary to use 250 ml of Aroclor 1254 in the jar to submerge the blades of the stirrer. Agitation for 0.5 h at 60°C and 1800 rev min⁻¹ produced a cloudy emulsion which was allowed to settle for 48 h, when the range of concentration was found to be 1–20 mg l⁻¹ and the emulsion became almost clear. This emulsion is referred to as type I. A second homogenization was carried out by transferring to a jar identical to the one used previously volumes of type I emulsion to produce emulsions of 10–300 µg l⁻¹, and stirring 1 h at 25°C and 1800 rev min⁻¹. This emulsion is referred to as type II. Type III emulsions were prepared by taking an appropriate volume of type I emulsion, adding it to a stainless steel blender jar to make a total volume of 500 ml, and homogenizing at high speed for 5 min.

All centrifugations were performed in a Beckman Model L3-50 ultracentrifuge at 20°C using SW 50.1 and SW 25.2 rotors.

The extraction procedure was that of Schoor (1973), with modifications of the ratio of water to hexane. Evaporation was carried out by placing the hexane extracts in a water bath at 35°C and allowing a gentle stream of air to blow across. This method was found superior to distillation in percentage recovery and time involved. When the extract volumes had to be reduced to less than 10 ml, dried, pre-purified nitrogen was used instead of air.

A Hewlett-Packard Model 5700 gas chromatograph with a linear electron-capture detector (⁶³Ni) was used for quantitative determination of the Aroclor 1254. The linearity of this detector eliminated use of different standards at each attenuation or reduction in volume of the sample, both being very time consuming and subject to errors. An OV-101 column (2% OV-101 on Gas Chrom Q, 100/120 mesh) was operated at 195°C with the detector at 300°C and the argon-methane (10:1) carrier gas at a flow rate of 25 ml min⁻¹. Except where noted, measurement was made by comparing total peak heights of sample and standard.

To determine the amount of Aroclor 1254 adsorbed on walls of the 34 ml stainless centrifuge tubes, the water phase was decanted and any adhering droplets removed with a disposable pipet. Since acetone injected with the sample was detrimental to the chromatographic column, a sonic probe and hexane were used for removal of Aroclor 1254 from the walls of the tubes. This was necessary because the thin layer of water remaining on the walls shielded the Aroclor 1254 and prevented it from being desorbed into the hexane phase. Sonification emulsified the water at the boundary layer, thus allowing the hexane to contact the adsorbed Aroclor 1254.

RESULTS

A typical chromatogram of an Aroclor 1254 standard in hexane (A) and a hexane extract of a type II emulsion (B) is shown in Fig. 3. Some of the 11 peaks indicated are multiple peaks. Only peaks 1–7 were used to calculate the "total" peak height on which all quantitations were based. Peaks 8–11 were excluded, because they were often too small to permit accurate calculations.

The effect of storage time on Aroclor 1254 emulsions of type I and type II is shown in Table 1. There is a fairly rapid initial decrease in Aroclor 1254 in all cases and it appears that a plateau is reached at around 7 µg l⁻¹. This should not be interpreted to

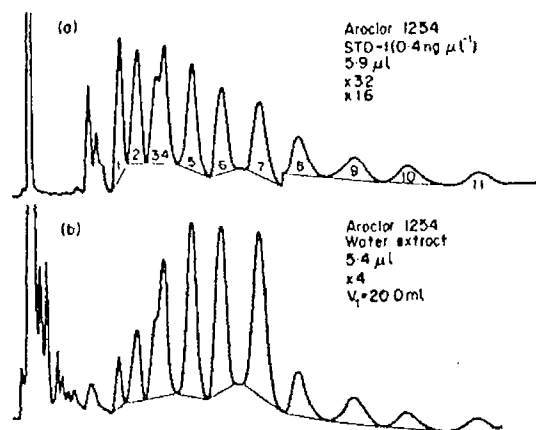


Fig. 3. Typical gas chromatograms (see text for detailed information).

mean that solubility is approached at that point, only that perhaps a stable emulsion is reached at that point.

The hexane extract of type II emulsion (chromatogram B) indicates a relative reduction in peak height for the early eluting peaks. This phenomenon is better described by the results shown in Table 2. For comparison peak 7 was arbitrarily assigned a relative value of 100%. The results indicate that on standing

a type II emulsion shows a reduction of the individual peaks, with the early eluting components, or less chlorinated biphenyls (Zitko, 1970), being reduced much more than the late eluting ones. The degree of reduction depends somewhat on the preparation and initial concentration of individual type II emulsions (Table 2). Type III emulsions of comparable "total" concentration show a relative distribution of the isomers identical to that of the standard.

The distribution of isomers in a hexane extract of the gill tissue of a pink shrimp (*Penaeus duorarum*) exposed to $2.5 \mu\text{g l}^{-1}$ Aroclor 1254 for 20 days is shown in parentheses at the bottom of Table 2. Because peaks 2, 4 and 7 showed obvious contamination, peak 6 was assigned the arbitrary, relative 100% value. The "total" concentration of 3.4 mg kg^{-1} was based on the total height of peaks 1, 3, 5 and 6, and on the wet weight of gill tissue (blotted to remove adhering water).

Filtration of type I emulsion through 450 nm (0.45μ) Millipore® filters revealed obstructed passage of Aroclor 1254 aggregates smaller than 450 nm. Starting with a 1 mg l^{-1} emulsion and changing filters after each filtration, less than $0.01 \mu\text{g l}^{-1}$ of the material remained in the water after 15 passages. Since aggregates in the starting emulsion were most likely smaller than 450 nm (calculations using equation (1)

Table 1. Effect of storage time on amount of Aroclor 1254 remaining in the water phase

Time (days)	$\mu\text{g/l}$ Aroclor 1254			
	Type I		Type II	
0	2300	301		50.2
2			286	
5		115		23.6
6		113		11.3
8		112		
9			123	
13		97	98.5	
15	502			
19	483	87	54.7	6.7
20			48.1	
21			44.5	
23		78		7.1
26				6.5
28	428			7.7
33	355			7.4
34	350			
41			15.5	
43	280			6.8

Table 2. Isomer distribution of Aroclor 1254 type II emulsion after standing for various periods of time in 3 l. glass bottle

Time (days)	Total conc. ($\mu\text{g/l}$) ¹	λ Peak Height ¹						
		Peak Numbers ²						
		1	2	3	4	5	6	7
2	286	76	93	95	95	98	104	100
9	123	79	78	89	94	98	99	100
13	98.5	79	79	93	98	99	96	100
19	54.7	72	75	85	93	91	95	100
20	58.1	64	70	80	89	92	94	100
21	44.5	61	65	82	90	99	93	100
41	15.5	37	41	56	70	77	88	100
21	13.4	16	27	44	55	76	87	100
33	3.6	12	21	39	45	64	82	100
38	1.6	9	10		31	46	63	100
	(3.4 ppm)	(41)		(80)		(87)	(100)	

¹Calculations are based on the relative height of peak 7 (see below).

²Peak numbers are shown on the chromatogram in Fig. 1.

DSW 025266

lead to roughly 10^{10} times the average molecular weight of Aroclor 1254, the Aroclor 1254 must have been adsorbed on the filter. This was also evidenced by the fact that the filter paper turned slightly transparent after the first passage during which about 95% of the material was removed from the emulsion.

The first centrifugation experiments were carried out by centrifuging 180 ml of $42 \mu\text{g l}^{-1}$ Aroclor 1254 type II emulsion in 60 ml polyacetate centrifuge tubes for 60 min at $107,000 \times g$ (maximum).

At an 85% total recovery the following distribution was found:

acetone extract of tubes	66%
hexane rinses of tubes	18%
top 50 ml water phase	5%
bottom 10 ml water phase	11%

The low recovery (85%) was probably due to incomplete extraction of the tubes in spite of refluxing with acetone.

Polyallomer® centrifuge tubes were tried next. When 180 ml of $286 \mu\text{g l}^{-1}$ type II emulsion were centrifuged in 60 ml Polyallomer tubes for 60 min at $107,000 \times g$ (maximum) the following distribution was found:

acetone extract of tubes	—
hexane rinses of tubes	22%
top 25 ml water phase	0.5%
bottom 35 ml water phase	0.6%

These percentages were based on the total amount of starting material, i.e. assuming 100% recovery instead of the 85% in the case of the polyacetate tubes. Extraction of the Polyallomer tubes by refluxing with acetone produced too many interfering peaks on the chromatogram, making complete recovery calculations impossible. Direct adsorption on Polyallomer

tubes was achieved by permitting type II emulsions to sit undisturbed in the tubes. Table 3 shows the outcome for two different concentrations.

To permit recovery and study of the material adsorbed on surfaces, 34 ml stainless steel centrifuge tubes were used for static tests, as well as for ultracentrifugal analysis. Table 4 shows the amounts of Aroclor 1254 adsorbed on the wall of a stainless steel centrifuge tube in relation to starting concentration and time. The amounts adsorbed from the 14 and $2 \mu\text{g l}^{-1}$ emulsions were greater than that adsorbed from the $113 \mu\text{g l}^{-1}$ emulsion during the same time period. It should be pointed out that 0.100 μg of Aroclor 1254 adsorbed as a monomolecular layer per tube represents about 2% of the minimum area available. The calculated inside area of a stainless steel centrifuge tube was 60.8 cm^2 . This area must be considered minimum because the surface was assumed to be ideally smooth, which certainly is not the case. However, for the approximations involved, this figure was used.

A simple calculation using equation (1) yields 0.613 nm^2 for the cross-sectional surface area of an average Aroclor 1254 molecule using the average molecular weight of 327 (Hutzinger *et al.*, 1972), and $\rho = 1.505 \text{ g cm}^{-3}$ (W. B. Papageorge, Monsanto Company, St. Louis, Missouri, personal communication). Utilizing a molecular model with the phenyl groups at right-angles to each other and bond length (Pauling, 1940) as the basis for calculations, a cross-sectional area of 0.643 nm^2 for the fully chlorinated and 0.356 nm^2 for the unchlorinated or biphenyl molecule was obtained. Values falling between are not linearly related to amount of chlorination. Using 0.613 nm^2 as an approximate, average cross-sectional area, 0.100 μg of Aroclor 1254 occupies 1.13 cm^2 in the form of a monomolecular layer. This corresponds to approximately $3 \mu\text{g l}^{-1}$ in a 34 ml stainless steel centrifuge tube. It can be seen that even at 50% adsorption from a $3 \mu\text{g l}^{-1}$ emulsion only about 1% (maximum) of the available surface area is occupied, and surface saturation was not a factor.

The amounts of Aroclor 1254 in the form of emulsions of type II and type III adsorbed on the walls of the stainless steel centrifuge tubes are shown in Table 5. There is a difference in adsorption of the two different types of emulsion in the absence of NaCl. At least for type III emulsions, the introduction

Table 3. Adsorption of Aroclor 1254 type II emulsion on Polyallomer centrifuge tubes on standing

Time (hrs)	Aroclor 1254 ($\mu\text{g/l}$) in water phase
0	125
3	86
72	3.3
0	45
1	35
3	27

Table 4. Adsorption of Aroclor 1254 on stainless steel centrifuge tubes as a function of time and concentration

Time (hrs)	Aroclor 1254 type II emulsion					% adsorbed
	Total (μg)	Water ($\mu\text{g/l}$)	Water (μg)	S.S. tube ¹ ($\mu\text{g/l}$)	S.S. tube ¹ (μg)	
0.5	3.83	113	3.63	107	0.18	5
1	3.83	113	3.31	97	0.30	9
2	3.83	113	3.20	94	0.33	13
16	3.83	113	3.14	92	0.51	16
1	0.48	14	0.35	10	0.08	23
2	0.06	2	0.03	1	0.02	67

¹Stainless steel centrifuge tubes.

Table 5. Adsorption of Aroclor 1254 on stainless steel centrifuge tubes

Time (hrs)	µg Aroclor 1254 ¹ adsorbed			
	Type II Emulsion 0 g/l NaCl	Type III Emulsion 30 g/l NaCl	Type III Emulsion 0 g/l NaCl	
0.5	0.19	0.09		
1.0	0.30	0.10	0.10	
2.0	0.33	0.14	0.14	
4.0	0.42	0.19		
19		0.39		
22			0.43	

¹Data adjusted to 4.00 µg total starting amount.

Table 6. Centrifugation of Aroclor 1254 in water of varying salinities at 69,000 × g (maximum)

Time (hrs)	µg/l Aroclor 1254 remaining in water phase ¹		
	0 g/l NaCl	15 g/l NaCl	30 g/l NaCl
0.5	13.9	7.1	6.0
1.0	12.5	6.6	4.9
2.0	7.2	4.6	2.9

¹Started with 50 µg/l Type III emulsion.

of 30 g l⁻¹ NaCl appears to have no effect on the amount of Aroclor 1254 adsorbed. However, centrifugation reveals a difference in the size of the aggregates formed in the presence of NaCl, as shown in Table 6.

In comparison with an Aroclor 1254 standard, the relative distribution of the isomers in emulsions of type II and III is quite different, as shown in Tables 7 and 8. However, in all cases the adsorbed Aroclor 1254 had a higher percentage of early eluting (gas chromatography) isomers than did that which remained in solution.

DISCUSSION

The original intent for conducting the work described was to find the absolute solubility of Aroclor 1254 in fresh and salt water. This, unfortunately, was not completely accomplished to any accurate degree, because a series of significant problems occurred at

the beginning of the centrifugation experiments. Recovery of Aroclor 1254 after centrifugation was low and, hence, led to the discovery that adsorption occurred on the walls of the polyacetate centrifuge tubes as well as on Polyallomer and stainless steel centrifuge tubes. Ultimately, only the stainless steel centrifuge tubes were used in the adsorption and ultracentrifugal studies.

The apparent disappearance of early eluting isomers, such as shown in Table 2, has been observed by others. It was found to occur in the eggs of the double-crested cormorant and regarded as possibly due to metabolic breakdown (Hutzinger *et al.*, 1972). Similar behavior in the carcasses of bobwhite quail after exposure to Aroclor 1254 was observed and believed to be because of isomeric transformations (Bagley and Cromartie, 1973). Application of Aroclor 1254 to different types of soil showed a reduced recovery of the early eluting, lower chlorinated biphenyls (Iwata, Westlake and Gunther, 1973), and it was pos-

Table 7. Distribution of isomers of Aroclor 1254 type II emulsion on standing in stainless steel centrifuge tubes

Storage (days)	Hrs in tube	µg/l	% Peak heights ¹						
			Peak number ²						
			1	2	3	4	5	6	7
1	0	310 water	93	90	98	99	98	100	100
5	0	115 water	53	71	73	91	98	98	100
	2	97 water phase	49	67	69	83	100	100	100
	2	12 adsorbed	96	106	103	127	119	100	100
8	0	112 water	51	67	71	82	96	97	100
	2	102 water phase	48	66	68	79	98	98	100
	2	8.0 adsorbed	69	82	85	104	107	100	100
13	0	97 water	47	64	68	81	97	98	100
	2	86 water phase	43	59	66	78	92	96	100
	2	6.1 adsorbed	47	68	77	94	101	98	100

¹Compared to standard Aroclor 1254 (Fig. 1). Calculations are based on the relative heights of peak 7.²Peak numbers are shown on the chromatogram in Fig. 1.

DSW 025268

Table 8. Distribution of isomers in the adsorbed fraction of Aroclor 1254 type III emulsion on standing in stainless steel centrifuge tubes

NaCl (g/l)	hrs in tube	water phase ($\mu\text{g/l}$)	adsorbed (μg)	% Peak heights ¹					
				Peak number ²					
				1	2	3	4	5	6
0	2	47.4	0.122	149	127	135	130	98	100
30	1	46.9	0.075	144	121	129	129	105	100
0	22	39.7	0.190	139	118	113	122	127	100

¹Compared to standard Aroclor 1254 (Fig. 1). Calculations are based on the relative heights of peak 6.

²Peak numbers are shown on the chromatogram in Fig. 1.

tulated that this may have been due to evaporation from the soil. My studies did not substantiate the observations by Zitko (1970) that when Aroclor 1254 emulsions are centrifuged the dissolved fraction is richer in the lower chlorinated biphenyls than is the original preparation. However, the difference could be due to the method of the preparation of his emulsion, which was similar to my type III emulsion. In both type II and type III emulsions the distribution of isomers in the water phase shows a loss of the lower chlorinated biphenyls on standing (Tables 7 and 8). This loss was accounted for in all cases by adsorption on the stainless steel centrifuge tubes, the "lost" lower chlorinated biphenyls always being found in the adsorbed fraction. Thus, at least from water emulsions of Aroclor 1254, loss of the lower chlorinated biphenyls is due to their relatively greater affinity for surfaces.

The published values for solubility of Aroclor 1254 in fresh and salt water of $2\text{--}3\text{ mg l}^{-1}$ and $1\text{--}1.5\text{ mg l}^{-1}$, respectively (Zitko, 1970), appear much too high. A conservatively high estimate based on my ultracentrifugal experiments indicates the average solubility of the isomers to be less than $0.1\text{ }\mu\text{g l}^{-1}$ for fresh water and approximately $0.04\text{ }\mu\text{g l}^{-1}$ (calculated from Table 6) in water containing 30 g l^{-1} NaCl. It is extremely difficult, in my opinion, to obtain an absolute value for the true solubility of the average molecular weight isomer of Aroclor 1254. The problem lies in the fact that at low concentrations, long centrifugation times (in excess of 12 h at $243,000 \times g$ (maximum)) theoretically are necessary to eliminate aggregates from the emulsion. At the low concentrations necessary to eliminate undesirable stirring back after completion of the centrifugation (Bowman, Acree and Corbett, 1960), adsorption on the walls of the stainless steel centrifuge tubes (67% at 2 mg l^{-1} for 2 h, Table 4) makes it all but impossible to employ ultracentrifugation for extended periods of time.

It appears that at least in the case of type III emulsions the adsorption from water emulsions containing 0 and 30 g l^{-1} NaCl was the same (Table 5), although the rate of sedimentation was quite different. The explanation for this lies in the fact that the size of the Aroclor 1254 aggregate is much larger in the presence of salt and, while this is not apparent at $1 \times g$, the larger aggregates are removed more quickly from the salt-containing emulsion during ultracentrifugation. This agrees very well with my hypothesis that

a larger aggregate is more stable under the given conditions and in the presence of salt, which is conducive to greater solute-solute (aggregate-aggregate) interaction.

SUMMARY

An extrapolation from the theory presented suggests that the use of "carriers" be continued with caution, because of two independent effects that may be present. One effect can most simply be described as an alteration of the aggregate-solvent interactions by "carriers" forming transition-like links between aggregates and solvent molecules. In such a fashion, solute aggregates are surrounded by "carrier" molecules, thus enhancing the ability of the aggregate to remain in a stable emulsion by permitting greater solute-solvent interaction. This can be illustrated graphically in Fig. 1 by enlarging region "B" over a greater range of aggregate sizes since some aggregates previously belonging to regions "A" and "C" now become more stabilized. It may also be visualized by flattening the two curves in Fig. 2, thereby extending their region of overlap. Thus, when added with a "carrier", more of an insoluble compound may be introduced into a stable water emulsion. The other effect may be due to possible interference with the uptake of a test compound by an organism. Any such uptake must by necessity be preceded by an adsorption to a surface of the organism such as the gills in a fish. If at this time the "carrier" molecules, which are located at the surface of the aggregate, affect the actual process of adsorption in any way, there will be a resultant change in the rate of transfer of the compound into the organism. If the toxicity is related to rate of uptake, there will be a concomitant change in toxicity.

This study shows, both theoretically and experimentally, that in so far as physical interactions are concerned, emulsions differing in degree of dispersion and stability can be formed, depending on the method of preparation and subsequent treatment. Consequently, the following questions should be answered before conducting bioassays in disperse aqueous systems.

- What are the solubility characteristics of the compound under investigation?
- To what extent are these characteristics related to field conditions?

(c) How can the solubility characteristics and field conditions be best simulated in the laboratory?

Such information would undoubtedly result in more precise and relevant data on acute toxicity as well as long-term effects regarding aqueous bioassay of water-insoluble test compounds.

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