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The need for field and laboratory studies of the physical and chemical behavior of environmentally significant organic molecules often poses problems because of the toxicity of the chemical and the difficulty of detecting it at environmentally significant concentrations. Two dysprosium tracers, dysprosium (III) trisacetylacetonate trihydrate and dysprosium(III) trisdibenzoylmethane monohydrate, were developed to mimic the physical and chemical behavior of toxic organic chemicals. Their stability in sea water and their bioconcentration behavior with the European oyster, Ostrea edulis were tested. A correlation between their n-octanol/water partition coefficient and bioconcentration factors was established. The behavior of dysprosium (III) trisdibenzoylmethane monohydrate as a Stable Activable Tracer for carbon tetrachloride was assessed in a laboratory experiment.

STABLE ACTIVABLE TRACERS FOR ENVIRONMENTALLY SIGNIFICANT ORGANIC MOLECULES

by

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INTRODUCTION

The development of agricultural and industrial chemicals and their use in environmental control has created a major environmental problem. Environmentalists have been increasingly aware of the seriousness of uncontrolled use and discharge of organic chemicals and a substantial effort is being made by private and federal institutions to assess the physical and chemical behavior of environmentally significant organic molecules (1, 2), their toxicity levels and the limitations for their use in environmental control (3, 4).

The direct assessment and analysis of these organic pollutants is a time-consuming and elaborate process (5). It usually involves extraction, separation and identification using chromatographic techniques (6-9).

An alternative approach to assess the behavior of organic pollutants in the environment would be to use tracers. Tracers (chemical, radioactive, and dyes) and more specifically Stable Activable Tracers have long been used in environmental studies (10). A Stable Activable Tracer (SAT) is a chemical tracer (metal chelate or stable non-radioactive nuclide) that one injects into the system under study followed by sampling and measurement of the tracer concentration by neutron activation analysis.

What Stable Activable Tracer to use in tracing environmentally significant organic molecules hinges on how it meets certain basic criteria:

(1) The tracer should have a low natural concentration in the system in which it will be used.

(2) The tracer should not be hazardous at the concentrations used.

(3) The tracer should be stable towards physical and chemical alterations.

(4) The thermal neutron capture cross section of the tracer nuclide should be appreciable.

(5) The product from the (n,γ) activation of the tracer nuclide should have a half-life long enough for easy counting.

(6) The tracer should show the proper physical and chemical behavior to model environmentally significant organic molecules.

In regards to point (6) above, we note that bioaccumulation of organic chemicals has long been associated with their non-polar nature. Measurements of the n-octanol/ water partition coefficient of such chemicals have long been a useful tool in predicting a certain trend in physical and behavioral patterns (11-13) and bioaccumulation (14-18). In choosing a Stable Activable Tracer to mimic the behavior of environmentally significant organic molecules, our first interest, therefore, would be in its n-octanol/ water partition coefficient (P). P should be of a comparable magnitude to the values of P for toxic organic chemicals. Metal chelates which have long been used in medicine (19) seem to

satisfy these requirements. They have the dual property of an organic moeity which confers to them a covalent character, bound to a central metal atom which could be detected by neutron activation analysis.

Of interest as candidates for Stable Activable Tracers are the rare earth derivatives of 1,3-diketones (20) with the following structural notation $(R\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH=\overset{\overset{O}{|}}{C}\text{-}R)_n M$ where n is the oxidation number of the central metal atom M.

They are attractive for the following reasons:

(1) The rare earth central metal atom in the chelate structure is easily detectable by neutron activation analysis. Table I gives some of the nuclear properties of the rare earth elements (21). Rare earth elements have sizeable thermal neutron capture cross-sections (europium σ = 5900 barns, dysprosium σ = 800 barns), their half-lifes are long enough for γ counting and the natural abundances of the target nuclides are high enough for easy detection. Most of the rare earth elements listed in Table I emit upon thermal neutron activation a measurable γ-ray with a high abundance.

(2) In natural waters (sea and river water), the rare earth elements have a low natural abundance (22-27) (see Tables II and III). In seawater, the abundance of rare earth elements ranges from 0.003 ppb for lanthanum to 0.000114 ppb for europium. In river water, their abundance is slightly higher but still much lower than transition metals (by a factor of 10^5):

TABLE I. NUCLEAR PROPERTIES OF THE RARE EARTH ELEMENTS (21)

Radionuclide	Stable Target Nuclide (% Abundance)	Thermal Neutron Cross-Section*	$t_{1/2}$
Pr-142	Pr-141 (99.99)	12.0	19.2 h
Sm-155	Sm-154 (22.53)	5.00	23.50 m
Eu-152	Eu-151 (47.77)	5900	12.7 y
Gd-159	Gd-158 (24.90)	3.4	18.00 h
Dy-165	Dy-164 (28.18)	800	139 m
Ho-166	Ho-165 (99.99)	64	66.78 m
Er-171	Er-170 (14.88)	9	7.52 h
Yb-177	Yb-176 (12.73)	7	1.900 h
Lu-176m	Lu-175 (97.4)	18	3.69 h

* in barns, 1 barn = 10^{-24} cm^2

TABLE II. ELEMENTAL COMPOSITION OF SEA WATER (22).

Element	Median	Range (part per billion)
Ag	0.29	0.055 -1.5
Al	2	0 -7
As	4	3 -6
Au	0.068	0.004 -0.027
B	4800	0.004 -5,290
Ba	20	5 -93
Be	0.00057	----
Bi	0.02	0.015 -0.033
Br	69,460	68,780 -70,180
Ca	425,200	423,000 -426,800
Cd	0.113	0.02 -0.25
Co	0.27	0.035 -4.1
Cr	0.3	0.23 -0.43
Cs	0.4	0.27 -0.33
Cu	2	0.2 -4
Dy	0.00073	0.00052 -0.0014
Er	0.00061	0.00066 -0.00124
Eu	0.000114	0.00009 -0.00079
F	1,340	1,280 -1,800
Fe	6.6	0.1 -62
Ga	0.03	0.023 -0.037
Ge	0.05	0.05 -0.06
Ga	0.00060	0.00052 -0.00115
Hg	0.03	----
Ho	0.00022	0.00012 -0.00072
I	63	48 -80
In	<20	----
K	412,000	406,000 -418,000
La	0.003	0.001 -0.006
Mn	1,340,000	1,330,000-1,340,000
Mg	1.5	0.2 -8.6
Na	11,100,000	11,070,000-11,140,000
Nd	0.0023	0.0013 -0.0065
Ni	5.4	0.43 -43
Pr	0.00064	0.00041 -0.00158
Pr	--	----
Se	0.2	0.34 -0.50
Si	2000	0 -4900
Sn	0.8	----
Sn	0.00042	0.00026 -0.0010
Sr	8,000	8,200 -7,600
Th	0.05	0.0002 -0.05
Ti	1	----
U	3	2 -4.7
V	2.5	2.0 -3.0
Zn	12.3	3.9 -48.4
Zr	--	----

TABLE III. ELEMENTAL COMPOSITION OF RIVER WATER (23-27).

Element	Median	Range (part per million)
Ag	0.00013	0.00001 -0.0035
Al	0.34	0.01 -2.5
As	0.0004	0.0004 -0.23
Au	0.00006	----
Ba	0.064	0.009 -0.15
Br	0.021	0.005 -140
Ca	15	4 -120
Cd	0.08	---
Cl	7.2	5 -35
Co	0.00050	0.0001 -0.006
Cr	0.00016	0.0001 -0.08
Cs	0.5002	0.00005 -0.0002
Cu	0.01	0.0006 -0.4
Fe	0.07	0.01 -1.4
Ga	0.001	----
Hg	0.00003	----
K	2.3	1.4 -10
Mg	4.1	1.5 -5
Mn	0.002	0.00002 -0.13
Mo	0.000035	----
Na	6.3	3 -25
Pb	0.005	0.0006 -0.12
Ra	3.9×10^{-10}	----
Rb	0.0015	0.001 -0.008
Se	0.02	----
Sn	0.00004	----
Sr	0.08	0.003 -0.8
Th	0.00002	----
Ti	0.0006	0.11
U	0.001	0.00002 -0.05
V	0.001	0.007
Zn	0.01	0.0002 -1
Zr	0.0026	0.0005 -0.022
Eu	0.000009	----
La	0.00033	----
Sn	0.000012	----
Dy	0.00017	----
In	0.00003	----

(3) Rare earths 1,3-diketones are very soluble in the common organic solvents and insoluble in water. In water, they give non-hydrolyzed solutions (20). Due to their solubility properties, they are expected to resemble several toxic organic chemicals.

(4) Rare earth elements are generally non-toxic at the concentration levels at which rare earth 1,3-diketones will be used in environmental studies. In Table IV, values of LD_{50} for rare earth elements are compared with the toxicity of selected environmentally significant organic molecules. Since the toxicity of rare earths 1,3-diketones could be also dependent on the organic ligand, the toxicity of acetylacetone used in the synthesis of rare earth acetylacetonates, is listed. Upon comparing LD_{50}'s of environmentally significant organic molecules to rare earth metals and acetylacetone as the organic ligand, one concludes that rare earth 1,3-diketones can be safely used at the intended concentration levels in environmental studies.

Keeping all the aspects of designing Stable Activable Tracers for environmentally significant organic molecules in mind, our objectives are as follows:

(1) Develop and prepare rare earth 1,3-diketones as potential Stable Activable Tracers for environmentally significant organic molecules.

(2) Test in the laboratory their physical and chemical stabilities in sea water.

TABLE IV. TOXICITY OF RARE EARTH ELEMENTS AND SELECTED ORGANIC CHEMICALS

Category	Chemical	LD_{50}[a]
Rare Earth Chlorides[b]	Lanthanum	4200
Rare Earth Nitrates[b]	Lanthanum	4500
	Cerium	4200
	Samarium	2911
	Europium	5000
	Dysprosium	3100
Toxic Organic Chemicals[c]	Paraoxon	3.5
	Parathion	5
	Dimethyl-Parathion	15
	DDT	17
	Endrin	18
	Acetylacetone[d]	1000

[a] LD^{50} = dose to kill 50% of the population.

[b] All LD^{50} are in mg chelate/kg body weight of laboratory animal - oral dose.

[c] All LD^{50} are in mg organic chemical/kg body weight of laboratory animal - oral dose.

[d] Organic ligand in rare earth acetylacetonates.

Reference: Toxic Substances List, U.S. Department of Health, Education and Welfare, National Institute for Occupational Safety and Health (1973 Edition).

(3) Measure their n-octanol/water partition coefficient.

(4) Measure their bioconcentration factor in oysters

(5) Apply the known structure/activity relationships to these tracers.

(6) Evaluate to what extent Stable Activable Tracers mimic environmentally significant organic molecules.

QUANTITATIVE STRUCTURE/ACTIVITY RELATIONSHIPS

Since the principles applied in choosing Stable Activable Tracers for environmentally significant organic molecules stem from known Quantitative Structure/Activity Relationships (QSAR), it seems appropriate to shed light on what is meant by QSAR.

Introduction

In this century chemists have become increasingly interested in the mechanism of reactions of organic molecules. In this regard, in 1935, L. P. Hammett proposed the Hammett equation (28):

$$\text{Log } K_x = \rho\sigma + \text{Log } K_H \qquad \text{(Equation 1)}$$

K_H is the ionization constant for benzoic acid in water at 25°C and K_x is the ionization constant for a meta and para derivative under the same experimental conditions. σ is a measure of electron withdrawing and electron donating meta or para substituents on the aromatic ring and ρ is a measure of the sensitivity of the ionization reaction to a change in solvent medium.

Since the Hammett equation is applicable only to meta and para derivatives of benzoic acids, other workers attempted to extend the findings of Hammett. Taft (29, 30) developed an empirical equation to relate steric effects and reaction mechanism of acid hydrolysis of esters. Charton (31) made a direct approach to define the steric hindrance of a substituent without defining any particular chemical reaction. An attempt was also made by Hancock and his co-workers (32)

Verloop, Hoogenstraaten and Tipker (33, 34) to institute a sharper definition of steric parameters.

Partition Coefficients and Π Values

Paralleling the efforts to relate chemical structure and physical parameters in organic molecules to their reaction medium under different experimental conditions, Meyer and Overton (35) were the pioneers to use the oil/ water partition coefficients as a means to define relative lipophilicity of biologically active organic compounds. Since then the oil/water partition coefficient of simple organic compounds have been correlated with biological uptake (1), lipophilic storage (36) and biomagnification (37).

The choice of a solvent system in which to measure the partition coefficient was based on the fact that an oil such as olive oil (35) or an alcohol would be best representative of a fatty phase in which the organic compound would be soluble. Since then, most correlations have been done using n-octanol/water partition coefficients (P), although it is by no means clear that n-octanol/water is the ideal solvent system for modeling all interactions of organic compounds with biological systems.

Although P can be used as a measure of the hydrophobicity of a whole molecule, one often works with a set of derivatives of a parent compound in which a large portion of the molecule remains constant. In this case, knowing the relative hydrophobicity of substituents can be enough for correlation analysis to enable one to work with the relative hydrophobicity of substituents. The parameter Π has been defined (38) analogous to σ in the Hammett equation:

$$\Pi x = \text{Log } P_x - \text{Log } P_H \qquad \text{(Equation 2)}$$

where P_x is the partition coefficient of a derivative and P_H that of the parent compound.

A compilation of Π values has been formed enabling one to calculate log P's from addition of Π values for parts of the molecule. The Π method of calculating partition coefficients has its advantages and drawbacks. They can be evaluated as follows:

(1) Inert groups such as $-CH_3$ have a relatively constant Π value, regardless of the electronic character of a second substituent. The halogens are somewhat more sensitive. Most sensitive are those substituents carrying a lone pair of electrons (e.g., OCH_3, NH_2, OH). In the latter cases, strong electron-withdrawing groups raise Π values, whereas electron-donating groups lower Π values.

(2) When two substituents are placed on an aromatic ring, the mutual electronic effect of one upon the other changes the Π value of each.

(3) Proximity effects of nearby substituents can affect Π values. In this case, the field effect appears to be much more important than the resonance effect in changing Πvalues.

(4) Simple addition of Π constants fails when strong electron interaction between substituents occurs (strong electron-donating and strong electron-withdrawing substituents on same ring).

The Fragment Method of Calculating Log P

To calculate log P values of organic molecules, one has the choice of summing up Π values (Π system) or by summing up the appropriate structual elements (fragment system). In the early work with Π calculations (14), erroneous values for a few aliphatic and aromatic hydrocarbons led to the conclusion that a different system for calculating log P had to be established. The fragment system is based on defining structural elements for molecules. Values for fragment constants were derived based on the definition that the Π constant of any substituent added to the fragment constant for hydrogen gives the fragment constant for that structure:

$$f_X = \Pi_X + f_H \qquad \text{(Equation 3)}$$

The fragment constant for hydrogen (f_H) was assigned a value of 0.23 based on the assumption that:

$$\tfrac{1}{2} \text{ Log P } (H - H) = f_H \qquad \text{(Equation 4)}$$

$$0.46/2 = 0.23$$

Thus, the fragment constants $f_H = 0.23$ and $f_C = 0.20$ become the only fundamental ones to calculate all alkane structures.

The fragment method of calculating log P retains constant fragment values for the fundamental structural elements and then looks for other factors (F) that affect the partitioning equilibrium in the more complex solutes where summation of fragments alone leads to spurious values:

$$\text{LogP} = \sum_1^n a_n f_n + \sum_1^m b_m F_m \qquad \text{(Equation 5)}$$

where a is the number of occurrences for fragment f of structural type n and b is the number of occurrences for factor F of character m.

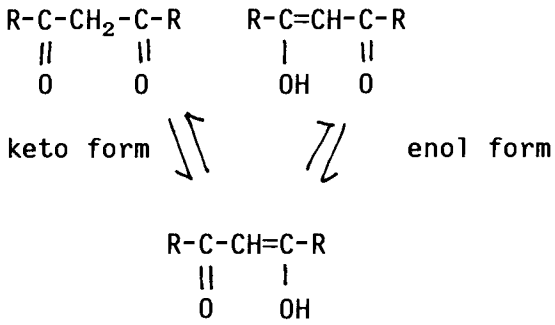
Log P values obtained by summing fragments and factors agree well with the experimental values obtained. In Appendix I, a detailed compilation of fragments is included to give a better insight on the use and amplifications of the Fragment Method of calculating log P.

EXPERIMENTAL

The overall plan for the research involves the synthesis, stability testing, partition coefficient measurements and bioaccumulation studies of rare earth 1,3-diketones for use as Stable Activable Tracers for environmentally significant organic molecules.

Tracer Preparation

Formation of Metal 1,3-diketones - The 1,3-diketones exist in two tautomeric forms:



The hydrogen of the hydroxyl group may be replaced by metals and a chelate ring will form owing to the marked coordinating power of the carbonyl oxygen.

Two dysprosium 1,3-diketone chelates were prepared, dysprosium (III) trisacetylacetonate trihydrate $Dy(acac)_3.3H_2O$ and dysprosium (III) trisdibenzoylmethane monohydrate $Dy(dbm)_3.H_2O$.

Dysprosium (III) trisacetylacetonate trihydrate

Acetylacetone: Reagent grade acetylacetone was extracted once with 10% ammonium hydroxide and twice with distilled water to remove any acetic acid. After drying over anhydrous sodium sulfate, it was distilled

through a Vigreux column and the fraction boiling between 139-140°C at 760 mm was collected for the synthesis.

Dysprosium Chloride: To a weighed amount of dysprosium oxide (purity 99.9%) was added enough concentrated hydrochloric acid and enough distilled water to yield a slurry which would give upon heating a clear solution containing dysprosium chloride. Excess hydrochloric acid was neutralized with 6N ammonium hydroxide to pH 5.

Acetylacetonate: The organic ligand was obtained by the addition of 18M ammonium hydroxide to an emulsion of freshly distilled acetylacetone in distilled water. Addition was done while constant stirring until the emulsion disappeared.

Synthesis: The synthesis of $Dy(acac)_3 \cdot 3H_2O$ was performed following standard procedures (39-41). Acetylacetonate in excess was added to dysprosium chloride solution while stirring at a very slow rate over a period of 2 hours. No precipitation was observed until the pH was adjusted to 6.4. The reaction mixture was left to stir overnight to insure complete reaction.

White crystals of $Dy(acac)_3.3H_2O$ were recrystallized twice with 95% ethanol. They were filtered, air-dried for 24 hours and sealed in tightly capped bottles (yield 35%).

The melting point measured on a Fisher-Jones melting point block was 136-138°C (reported 134-137°C).

Dysprosium (III) trisdibenzoylmethane monohydrate

Dysprosium Chloride was prepared as for $Dy(acac)_3.3H_2O$. Excess hydrochloric acid was neutralized with ammonium hydroxide to neutral pH.

Synthesis: The synthesis of $Dy(dbm)_3.H_2O$ was performed following standard procedures (42). To dysprosium chloride solution was added excess dibenzoylmethane in 95% ethanol. The suspension was stirred constantly and 1M ammonium hydroxide was added dropwise for a period of 2 hours. Orange yellow crystals of $Dy(dbm)_3.H_2O$ were obtained and recrystallized twice in 95% ethanol. The product was precipitated by further cooling. The extracted product was dried at 60°C for 24 hours (yield 30%). The melting point measured on a Fisher-Jones melting point block was 260-262°C (reported 261-265°C).

Analysis of Tracer Dysprosium Content

To measure the dysprosium content (gm dysprosium/gm tracer) and to compare it with the calculated value based on the molecular structure, samples of both tracers were analyzed by neutron activation analysis of the dysprosium metal in the chelate structure. Dysprosium is easily detected by neutron activation analysis due to its high thermal neutron cross section (σ = 800 barns) and the high natural abundance (28.18%) of the parent stable isotope dysprosium-164.

Three $Dy(acac)_3 \cdot 3H_2O$ samples ranging from 30 mg to 50 mg were accurately weighed. They were sealed along with dysprosium standards (8.69 mg Dy/ml 2N ultrapure HNO_3) in 2/5 dram polyvials and double encapsulated in 2 dram polyvials. The polyvials were previously cleaned following the procedure outlined in Appendix II. Samples and standards were activated simultaneously at a flux of 1.4 x 10^{11} neutrons/cm^2 sec for 4 minutes in the pneumatic terminal of the OSU TRIGA reactor. At the end of bombardment, each sample/standard was transferred to a clean

Table V. DYSPROSIUM CONTENT DETERMINED BY NEUTRON ACTIVATION ANALYSIS

Tracer	gm Dy/gm tracer		
	Calc.	Exp.	% Error
$Dy(acac)_3.3H_2O$	0.320	0.318 ± 0.005*	0.625
$Dy(dbm)_3.H_2O$	0.191	0.193 ± 0.002*	1.09

*Average of three determinations.

2 dram polyvial. Samples and standards were counted for the 94.5 kev photopeak of dysprosium-165 with a 40 cc Ge(Li) detector coupled with an ND600 multichannel analyzer. The same counting geometry was adopted (source at 10 cm from detector) for the samples and standards. The dysprosium content in the samples was determined by comparing the number of counts in the 94.5 kev photopeak at end of bombardment in both samples and standards.

Three $Dy(dbm)_3 \cdot H_2O$ samples were similarly activated. The sampling technique was slightly different. Dysprosium oxide (99.9% pure) was used as a standard. Samples and standards were weighed accurately and tightly packed in 2/27 dram polyvials, then double encapsulated in 2 dram polyvials. Samples and standards were activated at a flux of 1.4×10^{10} neutrons/$cm^2 \cdot sec$ for 3 minutes each. They were handled and counted similarly as the $Dy(acac)_3 \cdot H_2O$ samples. Results for the dysposium content are shown in Table V.

Laboratory Studies

Tracer Stability

In assessing the stability of the two tracers in sea water, it was impossible to directly activate the sea water samples containing nanogram levels of dysprosium tracers. Such an activation would have yielded a high background of sodium-24 ($t_{1/2}$ = 15 h) making it impossible to assay for the short-lived dysprosium-165 ($t_{1/2}$ = 139 m) present in trace amounts. Hence, an extraction step was necessary to minimize sodium contamination. N-octanol was chosen as the extraction solvent

for that step. Both tracers are very soluble in n-octanol which is the solvent of choice for partition coefficient measurements.

From a radiation stability standpoint, n-octanol is safe for neutron activation analysis. All organic compounds undergo radiolysis or breaking down of the molecule with a release of hydrogen gas. Molecular breakdown is induced by energy absorption. The most important source of energy during activation comes from the photon flux in the reactor core. The photon dose at 1 megawatt power (flux = 5×10^{12} neutrons/cm^2 ·sec) in the rotating rack of the OSU Triga reactor for an irradiation time for one half-hour is estimated to be 10^7 roentgen (5.46×10^{20}eV)*. Such a photon dose will be absorbed thus causing radiolysis and release of hydrogen gas. The rate of formation of hydrogen gas is characterized by its radiolysis yield G which is defined as the number of molecules of gaseous product per 100 electron volt of energy absorbed (43). Release of hydrogen gas can produce a high pressure buildup thus causing the activation vials to burst. G values give an insight on the extent of pressure buildup during irradiation, and their magnitude is affected by how much oxygen is present.

In Table VI, G values for hydrogen gas production are listed. A G value of 2.20 molecules/100 eV is attributed to a water solution containing cerous ions and is considered non-harmful upon irradiation. Aerating such a solution would lower the G value as shown in the case of ethanol. Deaerated pure n-octanol has a G value of 3.50 which would decrease upon aeration by a factor of 2, hence yielding a G value of approximately 1.7 to 1.8 which is significantly smaller than the 2.20 G value for cerous ion aqueous solution.

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* Measured by the staff of the OSU Triga reactor.

TABLE VI. RADIOLYSIS YIELD OF HYDROGEN GAS PRODUCTION, γ RAY IRRADIATION (44).

Sample	GH_2[a]	Pressure[b] of Hydrogen Gas
Pure water	0.40	0.0856
Water with cerous ion	2.20	0.470
Pure ethanol, deaerated	4.87	1.042
Pure ethanol, air saturated	1.73	0.1236
Pure n-octanol, deaerated	3.50	0.0204

[a]In molecules product/100 electron volt energy absorbed.

[b]Pressure of hydrogen gas resulting from a γ ray irradiation of 5.46×10^{20} eV. Pressures in atmosphere are calculated from the ideal gas law.

Resulting pressure due to hydrogen gas release when samples are irradiated with a photon flux of 5.46×10^{20}eV and at a reactor temperature of 15°C can be calculated from the ideal gas law assuming that hydrogen released is an ideal gas. Pressures are listed in Table VI. The maximum pressure that an irradiation vial (2/5 dram) can withstand without bursting is 2 atmospheres which is greater than the observed pressures when one activates water solutions or even n-octanol solutions.

Consequently, relative to trace element standards approved for neutron activation analysis, n-octanol does not constitute a hazard due to excessive pressure buildup of hydrogen gas. Hence, it was used for pick-up of the organically bound dysprosium from the sea water matrix and in partition coefficient measurements.

With acetone as tracer carrier: The two tracers were tested for stability at 25°C. Stock solutions of Dy $(dbm)_3.H_2O$ at concentrations of 4.0 ± 0.10 ng/ml and 2.0 ± 0.10 ng/ml were prepared in a matrix of $27^{o}/oo$ sea water at $10^{o}/oo$ in acetone. At the time intervals shown in Table VII, 1 ml aliquots of sea water were withdrawn from the solutions and evaporated under a gentle stream of hot air. The tracer deposit was dissolved in 2 ml of n-octanol of which 1 ml was prepared for neutron activation analysis along with dysprosium standards in the Lazy Susan of the OSU Triga reactor. Results of the ten day stability experiment are tabulated in Table VII.

The stability of Dy $(acac)_3.3H_2O$ was assessed similarly at an initial concentration of 10 ± 0.10 ng/ml. Two samples were only taken, the first and the tenth day. They were analyzed similarly and no signi-

Table VII. STABILITY OF $Dy(dbm)_3 \cdot H_2O$ AT 25°C IN SEA WATER AT $10^{o}/oo$ IN ACETONE.

Time (days	Initial Concentration 4.0 ± 0.10 ng/ml	Initial Concentration 2.0 ± 0.10 ng/ml
1	3.80 ± 0.143	2.00 ± 0.102
2	4.03 ± 0.101	2.05 ± 0.059
4	4.03 ± 0.123	1.81 ± 0.066
6	4.04 ± 0.114	1.84 ± 0.083
8	4.04 ± 0.120	1.94 ± 0.077
10	3.95 ± 0.150	1.85 ± 0.067

ficant change in concentration was observed (tenth day concentration: 9.90 ± 0.121 ng/ml).

Without acetone as a tracer carrier: To assess the effect of temperature and acetone as a tracer carrier, the stability of the two tracers was tested without acetone at 15°C. The two tracers were dissolved to saturation in distilled water and two stock solutions in sea water were made up containing 58.9 ng/ml Dy $(dbm)_3.H_2O$ and 17.40 ng/ml Dy $(acac)_3 \cdot 3H_2O$. 1 ml aliquots were withdrawn the 1st and the 12th day for both tracers. They were extracted and analyzed similarly. Results are shown in Table VIII.

N-octanol/water Partition Coefficients

By definition, the partition coefficient of a solute between two immiscible solvents is the ratio of its equilibrium concentration in each solvent (45). Occasionally, the ratio of solubilities in two separate solvents has been measured and reported as a partition coefficient (45, 46).

Hopkins and Douglas (47) have measured the partition coefficients of metal acetylacetonate between water and organic solvents and their solubilities. From their data, the measure of partition coefficient as a ratio of solubilities does not differ much from the direct measure of partition coefficient as a ratio of solute concentrations at equilibrium. The n-octanol/water partition coefficient for the dysprosium chelates was determined by measuring their solubility in n-octanol and water.

Reagents: Reagent grade n-octanol was purified by washing with (a) 0.1 N sulfuric acid and 0.1 N sodium hydroxide two times, (b) ultra-pure

TABLE VIII. STABILITY OF DYSPROSIUM TRACERS AT 15°C IN SEA WATER.

Tracers	Initial Concentration	12th Day Concentration
Dy $(dbm)_3.H_2O$	58.9	57.5 ± 3.15
Dy $(acac)_3.3H_2O$	17.4	17.1 ± 2.17

Concentrations are expressed in ng dysprosium tracer/ml of sea water.

water until neutral. It was dried over anhydrous calcium chloride and distilled twice through a vigreux column. Distilled, deionized and organic-free water was used for the water solubility measurements.

N-octanol and water in two separate flasks were saturated with dysprosium tracers by shaking at 20°C for a period of 24 hours. The solutions were then filtered, and the filtrates were centrifuged for 20 minutes at 10,000 rpm. One ml of each filtrate were pipetted in 2/5 dram cleaned polyvials, double-encapsulated and prepared along with dysprosium standards for neutron activation analysis at a flux of 5 x 10^{12} neutrons/cm^2 for 30 minutes in the Lazy Susan of the OSU Triga reactor. Samples were counted with a 40 cc Ge(Li) detector and the ND600 multichannel analyzer for dysprosium-165. The amount of dysprosium present was calculated by comparing activities at end of bombardment in the standards and samples. The amount of tracers was determined by correcting for the dysprosium ratio shown in Table V (0.191 for $Dy(dbm)_3 \cdot H_2O$ and 0.320 for $Dy(acac)_3 \cdot H_2O$). Solubility results are summarized in Table IX.

Bioconcentration Experiments

Frequently, partition coefficients have been found to correlate with bioconcentration factors of organic chemicals. Neely and coworkers (13) have found the bioconcentration factors of several chemicals in trout muscle to correlate in a straight line relationship with their partition coefficients. An equation of the straight line of best fit was determined and used to predict the bioconcentration factors of other chemicals from their partition coefficients.

TABLE IX. SOLUBILITY AND N-OCTANOL/WATER PARTITION COEFFICIENTS OF THE DYSPROSIUM TRACERS.

	Solvent[b]		
Tracer	Octanol	Water	LogP[b]
$Dy(acac)_3.H_2O$	34.44	1.742	1.296
$Dy(dbm)_3.H_2O$	6.176	0.0062	2.998

[a]Solubilities are in mg tracer/ml solvent;
[b]LogP as Log $\frac{\text{solubility in octanol}}{\text{solubility in water}}$

TABLE X. BIOCONCENTRATION FACTOR (BF) OF SELECTED PESTICIDES IN OYSTERS

Pesticide	Log BF	Log P*	Reference
Toxaphene	4.18	5.30	48
Dieldrin	3.46	4.20	48
Endrin	3.44	4.10	48
Heptachlor	3.92	5.80	48
Hexachlorobiphenyl	5.20	6.72	49
Methoxychlor	3.76	4.30	50
DDT	4.84	6.19	51

*From Hansch, C., Leo, A. J. "Substituent Constants for Correlation Analysis in Chemistry and Biology" Wiley Int. N.Y. (1979).

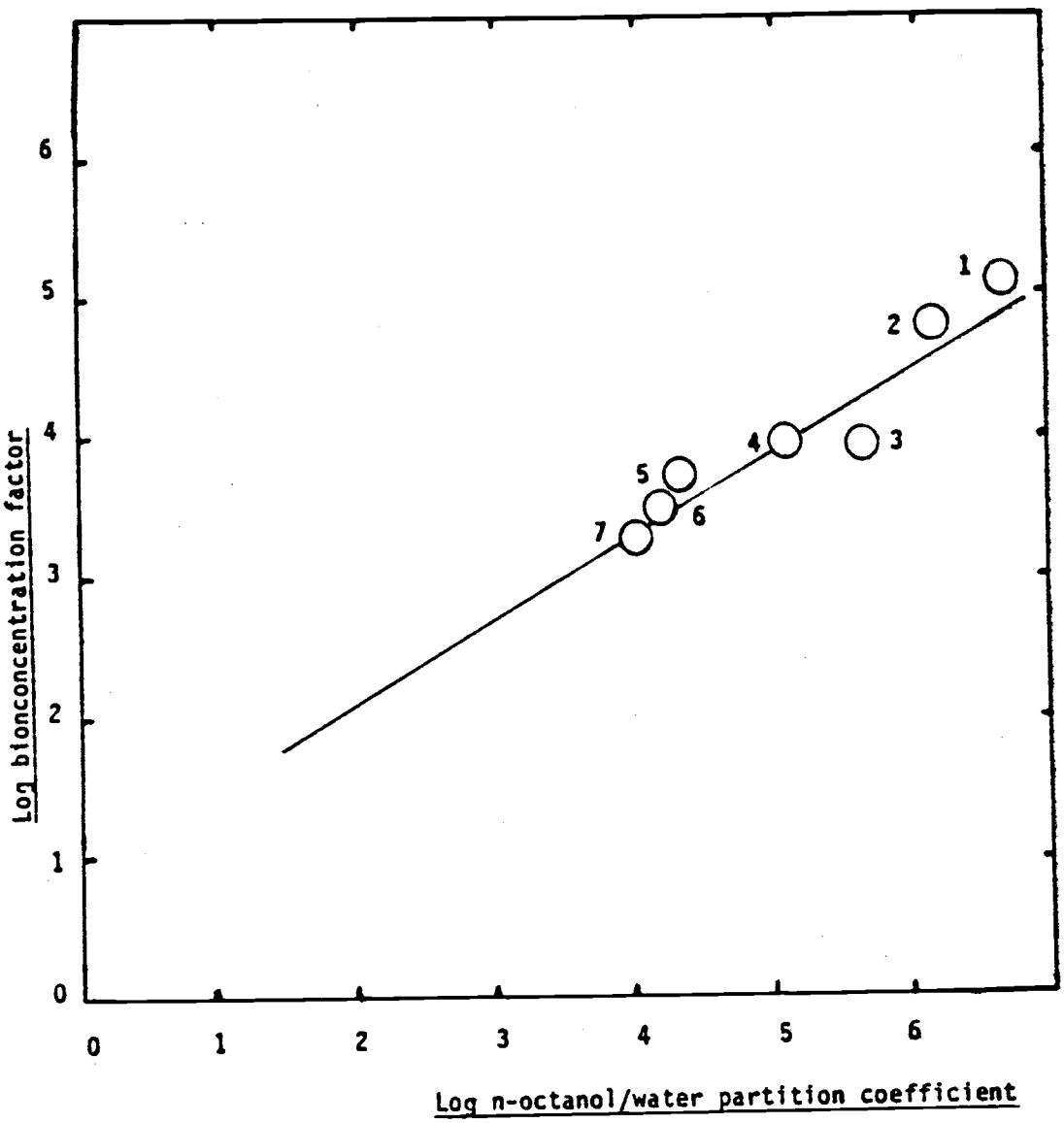

Figure I. Linear regression between logarithms of partition coefficient (P) and bioconcentration factor (BF) of selected pesticides in oysters. Compounds plotted are (1) Hexachlorobiphenyl (2) DDT (3) Toxaphene (4) Heptachlor (5) Methoxychlor (6) Dieldrin (7) Endrin.

Regression equation for best straight line fits:
Equation 6: $BF = 0.9906 + 0.5978 \text{ Log } P$, $R^2 = 0.9280$.

To test the bioconcentration behavior of the dysprosium tracers, the European oyster Ostrea edulis was chosen as a model system due to its ease of analysis and slow metabolism rate. As a baseline, the relatively meager data correlating bioconcentration factors in oysters and partition coefficients of organic chemicals was collected (Table X). The best straight line fit was determined (Figure I, Equation 6) and will be used to correlate the n-octanol/water partition coefficient of the dysprosium tracers with their bioconcentration factor.

Several attempts have been made to institute a universal definition of "bioconcentration" and "bioconcentration factor." In general, bioconcentration factor is defined as a ratio of any chemical residue in the organism to the residue in the ambient environment. This ratio is affected by many parameters to be defined for each experiment. The time factor is an important criterion to consider since "bioconcentration" is an equilibrium process similar to partitioning of a solute between two immiscible phases. In the case of measuring the bioconcentration factor in oysters or trout muscle, one would be measuring a "live organism partition coefficient" in which the body tissue of the orgranism is the lipid phase and the ambient water is the aqueous phase.

Dysprosium tracers

To measure the bioconcentration factor of the dysprosium tracers, oysters of comparable size (5 cm in diameter) were chosen. To expose the whole body tissues of the oysters and speed up the uptake process, the shell was cut so as to expose the gills and the cloaca (Figure II and III). The normal flow of water is from the gills to the cloaca

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Determined using Prophet.

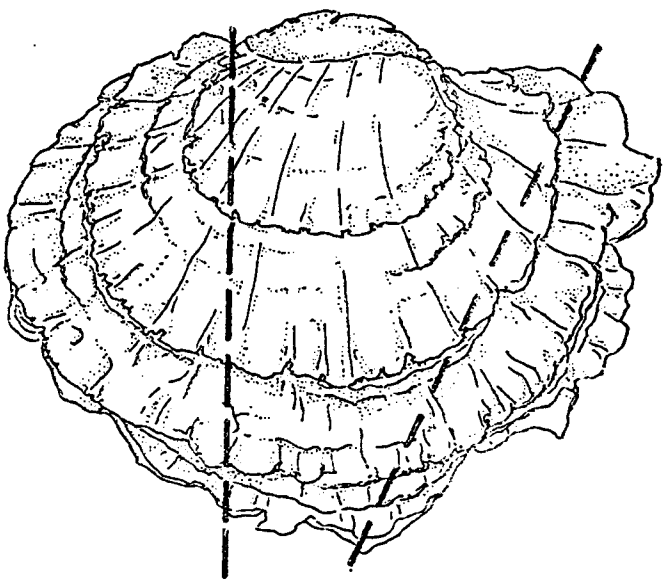

Figure II. The European oyster, Ostrea edulis, actual size. Incision of the left valve without damaging the mantle (dotted lines). Reproduced from reference 52.

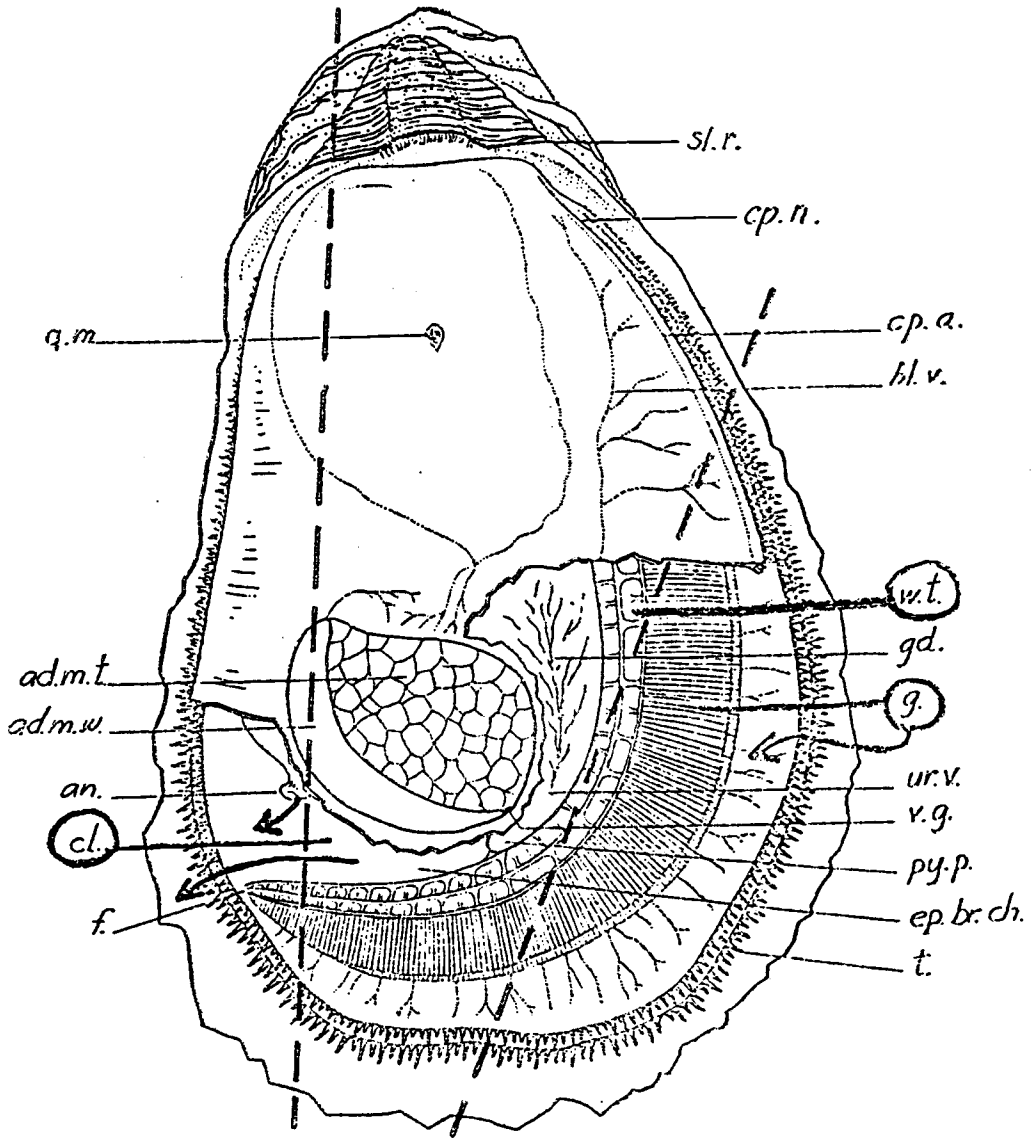

Figure III. The European oyster, Ostrea edulis, whole body. Incision along dotted lines to expose the gills (g) and cloaca (cl). Normal flow of water (f) from the gills to the cloaca through the water tubes (wt.).
Reproduced from reference 52.

through the water tubes (52). Incision of the left (lower) valve was done without damaging the mantle or breaking any tissues. The oysters were left to adapt for a period of 3-4 days during which shell growth at the point of incision was observed.

Four stock solutions containing 60 µg/l and 6 µg/l of the dysprosium tracers were prepared with 27% sea water at $10^{o}/oo$ acetone. The oysters with their shell cut were exposed for 5 days with no noticeable toxic effect, then they were removed and placed separately in fresh sea water. They were left to equilibrate for 12 days and then sacrificed for analysis.

Due to the high mineral content of the oyster tissues (sodium, magnesium, iron) which would give long-lived activation products of high abundance and would shield the low level activity of dysprosium-165, it was necessary to separate the organically bound dysprosium from the oyster tissue. A pre-activation separation procedure was devised. The organically bound dysprosium tracers were extracted by blending for 20 minutes the whole-body tissues with calcium chloride and 50 ml acetone in which the dysprosium tracers are very soluble. The resulting acetone extract was filtered and evaporated. N-octanol (2 ml) was used to pick up the residue which contains the organically bound dysprosium. 1 ml of the n-octanol extract was double-encapsulated and prepared for neutron activation analysis. The sea water (5 ml aliquots) was sampled as above and background samples (extract of non-exposed oysters) were prepared. Samples and standards (8.64 µg/l Dy/ml 2N ultrapure HNO_3) were activated for 1 hour at a flux of 5×10^{12} neutrons/$cm^2 \cdot sec$.

TABLE XI. BIOCONCENTRATION FACTOR (BF) FOR THE DYSPROSIUM TRACERS

Tracer	Log P	Log BF	
		Exp.	Calc.*
$Dy(acac)_3 \cdot 3H_2O$	1.296	1.686	1.765
$Dy(dbm)_3 \cdot H_2O)$	2.998	2.106	2.778

*Obtained from regression equation: Log BF = 0.9906 + 0.5978 Log P.

Table XI shows the bioconcentration factors obtained for both tracers. Both factors were corrected for efficiency (47.7%) which will be discussed below.

Efficiency of extraction

To determine the efficiency of extracting the organically bound dysprosium with acetone, oysters were exposed to europium (III) trisacetylacetonate trihydrate which is similar in structure and solubility properties to the dysprosium tracers. The oysters were prepared in a similar fashion and sacrificed for analysis in two groups. One group of oysters was extracted in acetone and the other group was activated directly after weighing. Samples were activated at a flux of 5 X 10^{12} neutrons/cm^2·sec for one-half hour. Samples were left to decay for 5 days to eliminate background interferences and were transferred to clean vials and counted for europium-152 ($t_{\frac{1}{2}}$ = 12.7 y) with a 40 cc Ge(Li) detector coupled with an ND600 multichannel analyzer. The efficiency was calculated by comparing activities at end of bombardment in the 121 KeV photopeak of europium-152 in the two groups of oysters. The measured efficiency was 47.70 ± 0.45%.

Dysprosium(III) trisdibenzoylmethane as Stable Activable Tracers for Carbon Tetrachloride

Based on the observations that the logarithms of bioconcentration factor and partition coefficient of selected pesticides correlate in a straight line fit, dysprosium(III) trisdibenzoylmethane monohydrate, log P = 2.998, and carbon tetrachloride log P = 2.83 (53) are expected to

bioconcentrate in a similar fashion in the European oyster, Ostrea edulis.

Carbon tetrachloride

Carbon tetrachloride (CCl_4) is a colorless liquid (b.p. 76°C) insoluble in water and miscible with most organic solvents. It is of general low insecticidal activity and is used for grain disinfectation when long exposures are possible (54). Its principal current use (more than 95%) is as a chemical intermediate in the production of fluorocarbons. Due to its high toxicity to humans upon inhalation or ingestion (lowest toxic concentration TCLo = 20 ppm) (3), the use of carbon tetrachloride as a household agent, industrial solvent, dry cleaning agent, and fire extinguishing agent has been banned by the U.S. Food and Drug Aministration (55).

The major discharge of carbon tetrachloride occurs during production, transport and storage, and use as a component of liquid grain fumigants in marine transportation (56). Carbon tetrachloride is found at trace levels in natural waters. Table XII gives estimates of carbon tetrachloride environmental concentrations. Such concentration levels in natural waters make carbon tetrachloride environmentally significant.

To this date, a relatively meager data on carbon tetrachloride and similar halomethanes has been compiled. No measurements of their residual concentration in marine organisms or biomagnification have been assessed.

TABLE XII. ENVIRONMENTAL CONCENTRATIONS OF CARBON TETRACHLORIDE

Location	Concentration	Reference
Marine Water		
Pacific Ocean	0.51 ± 0.28	57
Northeast Atlantic	0.17 ± 0.04	58
Atlantic Ocean	0.41	59
Precipitation and Fresh Water		
Rain (La Jolla, California)	2,800	57
Snow (North America)	300 - 2,000	57
Rain Water	18,000 - 20,000	60
Finished Drinking Water	2,000 - 3,000	61

Concentrations are in µg/l.

The bioconcentration factor of carbon tetrachloride in the European oyster, Ostrea edulis has been measured in a similar fashion as the dysprosium tracers.

Carbon-14 labeled, carbon tetrachloride with a total activity of 250 microcuries (9.40×10^6 disintegration/sec) was obtained in a sealed vial from New England Nuclear®. The total volume was 5 µl. The vial was broken and carbon tetrachloride was rinsed with 5 ml of acetone (Solution A). 1 ml of the above solution was pipetted into 1,000 ml $27^{o}/oo$ sea water. The initial exposure concentration was 1 µl carbon tetrachloride in 1,000 ml 27 $^{o}/oo$ sea water at $1^{o}/oo$ in acetone.

The oysters were prepared as above and exposed for 96 hours, at the end of which they were sacrificed and prepared for liquid scintillation counting (LSC) of carbon-14. The oysters were removed from their shell, air-dried, bagged in LSC bags and freeze-dried in liquid nitrogen. A mortar and pestle was used to crush the whole body which was then transferred to previously weighed LS vials. The whole bodies of the oysters were weighed (0.0251 g, 0.0213 g, 0.0191 g), then solubilized with 5 ml Triton-X-100* which is a non-ionic surfactant and has a quenching effect on carbon-14. 10 ml Aquasol** which is a universal LSC cocktail (fluor) added.

1 ml sea water aliquots were pipetted into LS vials and 15 ml fluor was added. To make up standards and measure the quenching of Triton-X-100 solution A was transferred to a 200 ml volumetric flask and enough

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Manufactured by:
*J. T. Baker Chemical Co.
**New England Nuclear

TABLE XIII. LIQUID SCINTILLATION COUNTING OF SAMPLES AND STANDARDS OF CARBON-14 LABELED CARBON TETRACHLORIDE

Standards		Triton-X-100 (ml)	Aquasol (ml)	Added Activity (dps)	
1		0	15	3.76×10^4	
2		1	14	3.76×10^4	
3		2	13	3.76×10^4	
4		4	11	3.76×10^4	
5		5	10	3.76×10^4	
Background					
1		0	15	None	
2		0	15	None	
Samples				**Activity (dps)**	**Amount**
Oysters	1	5	10	3870	0.0251 gm
	2	5	10	3526	0.0191 gm
	3	5	10	3469	0.213 gm
Sea Water	1	None	15	525	1 ml
	2	None	15	416	1 ml

"cold" carbon tetrachloride was added to a total volume of 200 ml (Solution B). Standards were prepared by changing the amount of quench added. 1 ml of Solution B was added to each standard.

Two background samples containing 15 ml of fluor and no activity were run. Table XIII gives a list of all the standards and samples run.

The samples and standards were counted for carbon-14 on a microprocessor controlled Beckman LS 7500. The measured bioconcentraton factor for carbon tetrachloride in the European oyster, Ostrea edulis was 355 (log BF = 2.550). The calculated bioconcentration from the regression equation is log BF = 2.682.

Dysprosium(III) trisdebenzoylmethane monohydrate and carbon tetrachloride

To assess the behavior of $Dy(dbm)_3.H_2O$ as a Stable Activable Tracer for carbon tetrachloride, oysters were exposed simultaneously to traces of dysprosium tracer and carbon tetrachloride. A stock solution containing 60 µg/l dysprosium tracer and 7 µg/l carbon tetrachloride was made up in 27% sea water at $1^{o}/oo$ in acetone as a tracer carrier. Oysters were exposed as described above and then sacrificed for analysis. Samples were analyzed similarly as was done previously then activated for one-half hour at a flux of 5×10^{12} neutrons/cm^2.sec. Samples were counted for dysprosium-165 and the bioconcentration factor was measured by taking the ratio of counts per second at end of bombardment in the oysters and sea water extracts. The measured bioconcentration factor of dysprosium(III) trisdibenzoylmethane monohydrate with traces of carbon tetrachloride in the European oyster Ostrea edulis was 2.191.

RESULTS AND DISCUSSION

Analysis of Tracer Dysprosium Content

The experimental value for the dyprosium content obtained by neutron activation analysis of accurately weighed amounts of dysprosium tracers agrees well with the calculated value based on molecular structure. Due to the difference in density and crystalline structure for both tracers, it was necessary to adopt two different sampling techniques. The density and crystalline shape can affect sample homogeneity and introduce experimental errors due to differences in activation and counting geometries. Dysprosium(III) trisacetylacetonate trihydrate posed no problems of geometry during counting and activation. Weighed amounts of tracer would pack easily at the bottom of the 2/5 dram polyvial used. Upon using the same vial size for dysprosium(III) trisdibenzoylmethane monohydrate, an observation was made that the tracer would not pack at the bottom of the 2/5 dram polyvial. The tracer would spread as a thin layer on the walls of the vial; such spreading being random and heterogeneous creates different geometries. Thus, smaller vials (2/27 dram) were used to eliminate geometry errors. Different standards were also used. A solid dysprosium oxide standard packed in 2/27 dram polyvial was used for $Dy(dbm)_3 \cdot H_2O$ and a liquid dysprosium standard in 2/5 dram polyvial was used for $Dy(acac)_3 \cdot H_2O$.

Tracer Stability

The tracers remained soluble and non-dissociated with and without acetone as a tracer carrier and at two different temperatures for a

period of 10 to 12 days. This property should enhance the attractiveness of the tracers for long-term tracer experiments.

N-Octanol/Water Partition Coefficients

The tracers n-octanol/water partition coefficients fall within the general range of a number of pesticides. Based on the observation that the logarithms of the n-octanol/water partition coefficient and the bioconcentration factors correlate into a straight line fit, one would expect that at equilibrium, chemicals with same log P should bioconcentrate similarly.

Bioconcentration Experiments

A correlation was drawn between logarithms of partition coefficients and bioconcentration factors of several chemicals in oysters. Log BF and log P of selected organic chemicals are well correlated into a straight line fit. This correlation will be used as a baseline to predict with a first approximation the bioconcentration factor of a chemical given its n-octanol/water partition coefficient.

The bioconcentration factor of the dysprosium tracers was measured under the assumption that one would be measuring a "live organism partition coefficient" where the oysters whole-body tissue is the organic phase and the ambient sea water is the aqueous phase. The oysters shell was incised to permit a free flow of water from the gills to the cloaca and speed the uptake and equilibrium process. The calculated value for the bioconcentration factors by substituting the values of log P in the regression equation agree well with the experimental value (Table XI).

The bioconcentration factors of the Stable Activable Tracers appear to follow the same systematic dependence on log P as do a number of environmentally significant organic molecules. Thus, it might be expected that these tracers would show similar bioconcentration behavior as organic analogs with similar values of log P.

Carbon tetrachloride with a log P of 2.83 is expected to bioconcentrate in a similar fashion as dysprosium(III) trisdibenzoylmethane monohydrate (log P = 2.998). The bioconcentration factor of carbon tetrachloride has been measured to ascertain its dependence on log P. Results of the analysis for carbon tetrachloride show that the experimental bioconcentration factor agree well with the calculated factor derived from the regression equation.

The oysters were exposed to both dysprosium tracer and carbon tetrachloride to ascertain the effect of carbon tetrachloride on the bioconcentration factor of $dy(dbm)_3 \cdot H_2O$. The bioconcentration factor of the dysprosium tracer was not affected significantly by the presence of carbon tetrachloride in trace amounts.

Hence, dysprosium(III) trisdibenzoylmethane monohydrate can be used as a Stable Activable Tracer for carbon tetrachloride.

COROLLARY

N-Octanol/Water Partition Coefficient and Aqueous Solubility

Paralleling the efforts to relate chemical structure and physical parameters such as the n-octanol/water partition coefficient, empirical equations have been established to relate the aqueous solubility of a wide variety of chemicals and their n-octanol/water partition coefficients. Chiou et al. established an empirical equation including aliphatic and aromatic hydrocarbons, aromatic acids, organochlorine and organophosphate pesticides, and polychlorinated biphenyls (15). The logarithms of the n-octanol/water partition coefficient (P) and the aqueous solubility (S) in μmole/l correlate into a straight line:

$$\log P = 5.00 - 0.670 \log S \qquad \text{(Equation 7)}$$

This correlation does not seem to apply for high-melting solids. Barnejee et al. tried to extend the above correlation to include high-melting solids (18).

$$\log P = 6.5 - 0.89 \log S - 0.015 (mp) \qquad \text{(Equation 8)}$$

Equation 8 is introducing a new element which is the melting point of (mp) in °C for solid solutes. For liquid solutes, 25°C was used instead of the melting point (62).

The use of Equation 7 does not differentiate between liquid and solid organic chemicals while Equation 8 is most accurate and accounts for those cases where Equation 7 could not be applied.

TABLE XIV. CORRELATION OF N-OCTANOL/WATER PARTITION COEFFICIENTS AND AQUEOUS SOLBILITY OF METAL CHELATES

Chelate	S	mp	Log P		
			Exp.	Eq. 7	Eq. 8
$Dy(acac)_3 \cdot 3H_2O$	3504.44	138	1.296	2.625	1.275
$Dy(dbm)_3 \cdot H_2O$	7.308	260	2.99	4.421	1.836
$Cr(acac)_3$	2579	213	1.838	2.714	0.27
$Co(acac)_3$	3652.3	216	2.18	2.613	0.0898
$Cu(C_9H_6NO)_2$	8.38	---	2.70	4.381	---

Eq. 7: $\log P = 5.00 - 0.67 \log S$

Eq. 8: $\log P = 6.5 - 0.89 \log S - 0.015 (mp)$
S in μmole/l, mp: melting point in °C

Application to Metal Chelates

How do Equations 7 and 8 apply in the case of the dysprosium tracers and in the general case of β-diketonates or metal chelates? In Table XIV, the dysprosium tracers, chromium and cobalt acetylacetonates and copper 8-quinolinate are listed with their solubility (S) and their melting points. Equation 8 seems only to apply in the case of $Dy(acac)_3 \cdot H_2O$. Equation 7 does not apply in any case. Hence, both equations which were meant to correlate solid and liquid organic solutes cannot be extended to metal chelates.

The Fragment Method of Calculating Log P

How can the Fragment Method of calculating log P be applied to metal chelates in the development of Stable Activable Tracers for environmentally significant organic molecules? The Fragment Method of calculating log P has a predictive power when applied to organic chemicals. A software package for calculating log P values from connection tables has been implemented and tested (63). The routine, named CLOGP, is based on the Fragment Method developed by Hansch and Leo. However, there are some limitations to the use of this program. It cannot handle any ionic, inorganic or organometallic compounds. Hence, it cannot be applied to metal chelates with the purpose of calculating their n-octanol/water partition coefficient.

CONCLUSION

Metal chelates appear to be successful candidates as Stable Activable Tracers for environmentally significant organic molecules. For a generalized approach to the design of Stable Activable Tracers, the predictive power of the Fragment Method of calculating log P will have to be extended to include metal chelates. For that purpose, we propose to tackle the following problems of measuring fragment constants for metals starting from experimental n-octanol/water partition coefficients. Once the CLOGP routine is expanded to include metal chelates, one would be able to draw structure/activity relationships applicable to metal chelates.

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APPENDICES

APPENDIX I

The Fragment Method of Calculating Log P

Abstracted from: Hansch, C., Leo, A. J., "Substituent Constants for Correlation Analysis in Chemistry and Biology." Wiley Interscience, N.Y. (1979).

Single atom fundamental fragment:

(a) -C- in CH_4 and >C in $H_2C{=}CH_2$

(b) -H in H-C≡C-H, but not in R-C(=O)-H or in >N-H

(c) -F in CH_3-F but not in R-SO_2-F

(d) -O- in CH_3-O-CH_3 but not in CH_3-C(=O)-O-CH_3

Two-atom fragments:

(a) C≡N (b) -C(=O)- (c) -S(=O)- (d) -OH

(e) >C=N- (f) >N-H

Multiple-atom fragments:

(a) -C(=O)-NH_2 (b) -NH-C(=O)-NH- (c) -NH-N=CH- (d) -NH-NH-

(e) -NH_2

Subscripts

1) On F indicate structure (may be in WLN if complex); for example:

(a) fMVM = f-NH-C(=O)-NH-

2) On F indicate type:

(a) F_b = bond factor.

(b) F_{cBr} = chain branch factor.

(c) F_{gBr} = group branch factor.

(d) $F_{=}$ = double bond factor.

(e) $F_{\equiv}$ = triple bond factor.

(f) F_{mhG} = multiple halogenation, germinal.

(g) F_{mhV} = multiple halogenation, vicinal.

(h) F_{p-1} = proximity factor, H-polar fragment, one carbon separation.

(i) F_{p-2} = proximity factor, H-polar fragment, two carbon separations.

3) Underlining any symbol means it is present in a ring.

Superscripts (on either f or F)

1) None = alphatic structural attachment.

2) ϕ = attached to aromatic ring; if bivalent, the attachment is from left as written.

3) $1/\phi$ = as 2 but attachment from right as written.

4) $\phi\phi$ = bivalent fragment, two aromatic attachments.

5) X = aromatic attachment, value enhanced by second, electronic withdrawing substituent ($\sigma_I > 0.35$).

6) IR = benzyl attachment (IR = $CH_2C_6H_5$ in WLN).

Calculations

Cyclohexene

LogP  $= 6f_C + 12f_H + (6-1)\ F_b + F_=$

$= 6(0.20) + 12(0.23) + 5(-0.09) - 0.05$

=	Calcd	Obsd
	2.96	2.86

Butane

LogP $CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_3 = 2fCH_3 + 2fCH_2 + (3-1)F_b$

$= 2(0.89) + 2(0.66) + 2(-0.12)$

=	Calcd	Obsd
	2.86	2.89

Cyclopentane

LogP  $= 5fCH_2 + (5-1)F_b$

$= 5(0.66) + 4(-0.009)$

=	Calcd	Obsd
	2.94	3.00

Benzene

LogP  $= 6fCH_2 + (6-1)F_b + 3F\underline{\phi}$

$= 6(0.66) + 5(-0.09) + 3(-0.42)$

=	Calcd	Obsd
	2.25	2.13

or:

LogP  = LogP  $+ 3F\underline{\phi}$

$= 3.44 + 3(-0.42)$

=	Calcd	Obsd
	2.18	2.13

APPENDIX II

POLYVIAL CLEANING PROCEDURE

(1) Wash polyvials with 2N nitric acid for 10 minutes using "ultrasonic" cleaner*.

(2) Rinse polyvials with distilled water for several times.

(3) Wash polyvials with absolute (95%) ethanol in "ultrasonic" cleaner for about 10 minutes.

(4) Drain the alcohol and wash with acetone for 10 minutes.

(5) Air dry polyvials on a clean sheet in "clean" room equipped with laminar flow filter.

*Manufactured by Branson Cleaning Equipment Co., Shelton, Connecticut, U.S.A.

APPENDIX III

List of Pesticides and Their Chemical Names

DDT

Chemical name: Dichloro diphenyl trichloroethane.

Action: Insecticide

Dieldrin

Chemical name: 1,2,3,4,10,10-hexachloroexo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphtalene.

Action: Insecticide.

Endrin

Chemical name: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphtalene.

Action: Insecticide.

Heptachlor

Chemical name: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane.

Action: Insecticide.

Methoxychlor

Chemical name: 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane.

Action: Insecticide.

Paraoxon

Chemical name: Diethyl 4-nitrophenyl phosphonic acid ester.

Action: Insecticide.

Parathion

Chemical name: 0,0-diethyl o-p-nitrophenyl phosphorothioate.

Action: Insecticide.

Parathion-dimethyl

Chemical name: 0,0-dimethyl o-p-nitrophenyl phosphorothioate.

Action: Insecticide.

PCB (polychlorinated biphenyls)

Chemical name: Mixture of chlorinated terphenyls.

Action: Insecticide, plasticizers.

APPENDIX IV

Copper 8-quinolinate

Copper 8-quinolinate seemed appropriate for consideration as Stable Activable Tracer for environmentally significant organic molecules. Preliminary experiments were run on copper 8-quinolinate to assess its stability at trace levels in sea water and its hydrophobicity in n-octanol/ water solvent system. No further experiments such as bioconcentration were run because of the high natural abundance of copper as a trace element in natural systems and in sea and river waters. Included in this appendix is the data obtained for preliminary experiments.

Structure

Copper 8-quinolinate is a metal chelate of formula $(C_9H_6NO)_2$. Copper is chelated by 8-hydroxyquinoline and the chelate is mostly used for crop protection (64). Generally, it is formulated and used as a dihydrate with a copper content of 0.18 gm copper per gm of chelate. It was obtained and used as such from Sigma® Chemical Company.

Detection of copper

Upon neutron activation, copper activates into 12.8 h copper-64. The thermal neutron cross section of 4.5 barns and the high natural abundance (69.10%) of the parent stable isotope copper-63 makes long-lived copper-64 easily detected by neutron activation analysis. Copper-64 is a positron emitter. Thus, it can be detected by gamma-gamma coincidence counting of both 0.511 MeV annihilation gamma rays (65). These gamma rays are emitted at 180° angle and can be detected simultaneously by two NaI(Π) detector.

Stability of copper 8-quinolinate in sea water

Two stock solutions containing 0.60 ± 0.05 µg/ml and 1.80 ± 0.10 µg/ml copper chelate were prepared by mixing and diluting weighed amounts of chelate in $27^{o}/oo$ sea water. Solutions were kept at room temperature for 7 days. Each day 1 ml aliquots were withdrawn and evaporated under a gentle stream of air. Copper 8-quinolinate residue was collected by washing the sodium chloride deposit with 2 ml n-octanol of which 1 ml was double-encapsulated and activated for copper-64. The extraction step was necessary to avoid contamination with long-lived sodium-24 ($t_{\frac{1}{2}}$ = 15 h) which emits a gamma ray at 0.511 MeV photopeak. To account for background interferences, 1 ml sea water used to make up the stock solution was evaporated, extracted and activated. The amount of copper present was calculated by reference to a standard containing 10 µg Cu/ml of 2N HNO_3. Samples and standards were activated at a flux of 5 x 10^{12} neutrons/cm^2·sec for one-half hour. They were left to decay for 6 hours then transferred to clean 2 dram polyvials and counted for coincident annihilation radiation. The coincidence counting system used is pictured in Figure IV. Any positron emitter present could be detected, hence the necessity of establishing decay curves for sample and standards and measure the decay rate of copper-64 based on its half-life. Samples and standards were counted for 10-100 seconds each at time intervals to cover 3 half-lives of copper-64. Counts at end of bombardment were extrapolated and used to assess the amount of copper present. Samples were corrected for background interferences present in the pure sea water extracts, and the amount of chelate present was

calculated making use of the copper content (0.18 gm copper/gm chelate). Under "natural" sea water conditions, copper 8-quinolinate at two concentration levels was found to be stable for a period of 7 days (7th day concentration: 0.55 ± 0.05 µg/ml and 1.70 ± 0.10 µg/ml).

Partition coefficient

Copper 8-quinolinate is highly soluble in lipids and insoluble in water; it has an n-octanol/water partition coefficient (Log P = 2.70) close to known toxic organic chemicals. No bioconcentration or toxicity data have been reported.

Conclusion

Further work on copper 8-quinolinate will be carried out. A priori, considering its high log P and stability in sea water, copper 8-quinolinate might prove to be a successful candidate for tracing environmentally significant organic molecules. For that purpose, an accurate separation technique needs to be developed to eliminate any interference due to the abundant inorganic copper present in natural systems.

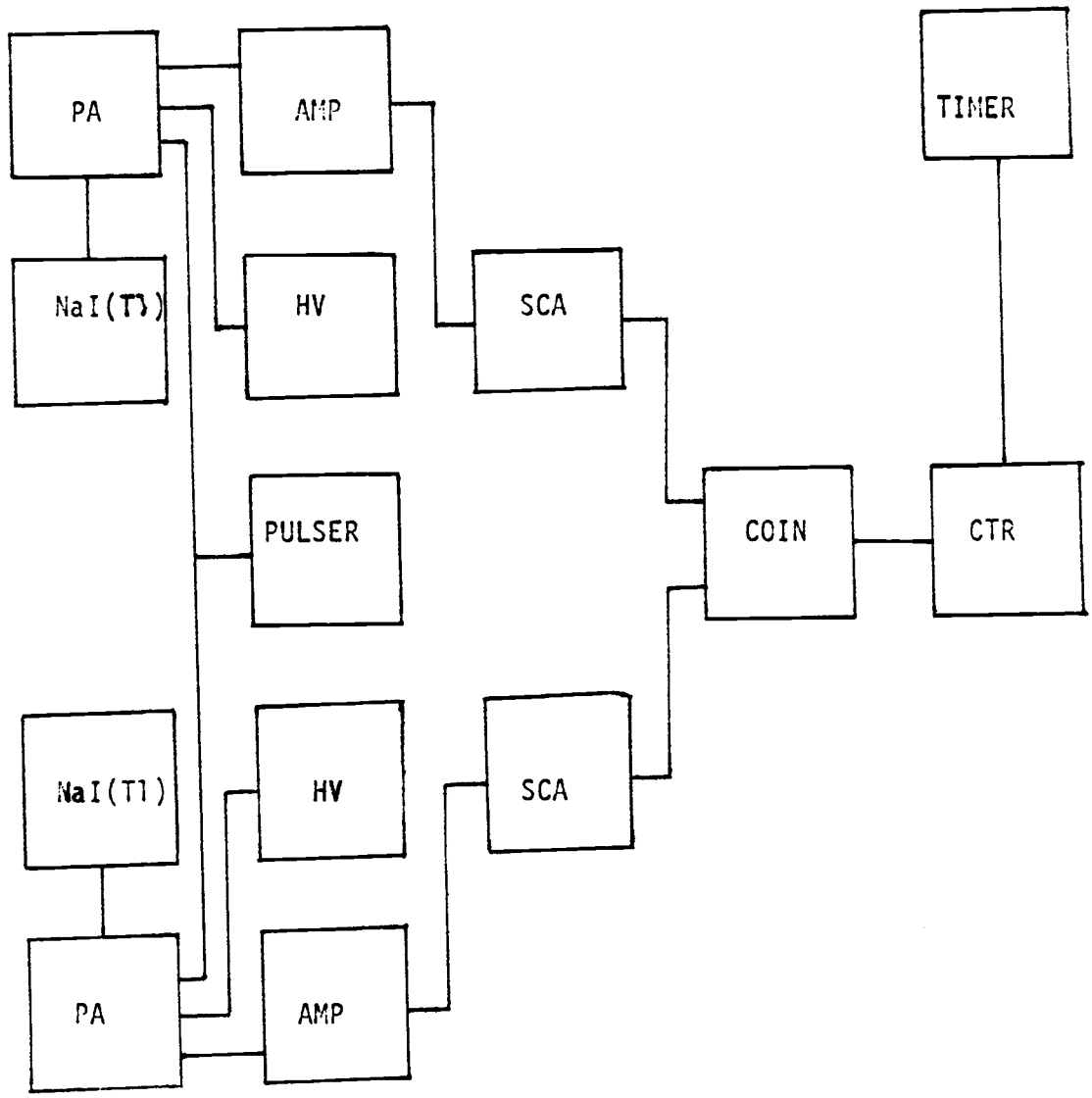

Figure IV. Coincidence system: Preamplifier (PA), NaI (Tl) detector, Amplifier (AMP), High Voltage Supply (HV), Pulser, Single Channel Analyzer (SCA), Coincidence Unit (COIN), Counter (CTR) and Timer.