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PREDICTING THE BIO-ACCUMULATION AND TOXICITY OF ORGANIC COMPOUNDS

Of the more than four million chemicals that are registered, approximately 63,000 are in "common" use (Maugh 1978). Until recently, few laboratories studied more than a few dozen of these constituents, but with the establishment of EPA's Priority Pollutant List (U. S. Environmental Protection Agency 1977), a growing number of wastewaters are now being analyzed for approximately 120 organic compounds. From a practical point of view this is still an unmanageable number and it is not sensible to expect researchers to look for all 120 compounds all the time. Therefore, the objective of this research was to determine if there was some way of establishing the relative potential for toxicity of the various compounds so that priorities could be established and attention could be focused on those compounds most likely to cause problems. By using a combination of n-octanol/water partition coefficients and available toxicity data we are able to predict bioaccumulation of synthetic organic compounds in marine animals and to rank these compounds in order of relative importance.

In this research we looked in sediments and animals collected in the Palos Verdes area for 27 compounds that had been identified in the Los Angeles County effluent. These 27 compounds were selected because they were present in highest concentrations in effluents and because they had a wide range of partition coefficients. We compared the effluent concentration to sediment and tissue concentrations and to the n-octanol/water partition coefficient to determine if any of these variables were related. In particular we wanted to find out if the n-octanol/water partition coefficient can be used to predict the bioaccumulation of organic compounds. It can.

METHODS

Sampling

From November 1980 to August 1981 we collected, quarterly, one-week composites for extractable organics and two sets of grab samples for volatile organics analysis (VOA) of final effluent from the Los Angeles County Wastewater Treatment Plant (JWPCP). Sediments were also sampled on a quarterly basis corresponding with the wastewater sampling dates, except the third quarter was missed due to mechanical failure of the boat used for sampling. Two grab samples for VOA and five replicate composite samples for extractable organics of surficial sediments (0-2 cm) were collected from station PV 3-1 (6 km NW of the discharge zone at a depth of 60 m) using a modified Van Veen sampling device. Biological samples were also collected from station PV 3-1 during June, 1981. Standard trawls were used to collect California halibut,

Pacific sanddab, Dover sole, scorpionfish, white croaker, ridgeback prawn, and red pointer crab. A 1 mm metal net on a sled was used to collect samples of small invertebrates from just above the bottom sediments. This net has an opening 75 cm wide by 15 cm high, the lower edge of which is 7 cm above the bottom runners. It was towed along the depth contour at a depth of 30 meters. The final sample, sorted under clean laboratory conditions, contained 74% (by weight) mysids and 23% decapod shrimp.

Analysis

All sample extractions were done either by using the EPA protocol (U. S. Environmental Protection Agency 1977) or by using previously reported methods (Young *et al.*, 1976), except for the extraction of sediments and tissues for Volatile Organics Analysis (VOA). These were extracted following EPA method 601, except that 3 ml of purged water per gram of sample was added to the sample vial which was then swirled to disperse the solids. Internal standards were added and the vial was swirled again and allowed to equilibrate. The screw cap and septum were removed and a stainless steel cap insert with an inlet tube and exit port was attached to the sample vial. The vial was immersed in an 80°C water bath and purged for 12 minutes with an inert gas at 40 ml/min. The trap was then heated to 200°C for five minutes and backflushed onto the GC/MS column.

Analysis by gas chromatography mass spectrometry (GC/MS) was conducted at California Analytical Labs of Sacramento, California, using a Finnigan 3200 GC/MS equipped with a 1.8 meter x 2 mm ID glass column packed with 0.2% Carbowax for VOA and an SE54 60 m fused silica capillary for extractable organics. Methods for gas chromatographic (GC) analysis by SCCWRP have been previously reported for pesticides and chlorinated benzenes (Young *et al.* 1976). Naphthalene and related compounds were analyzed by a Varian 5600 Liquid Chromatograph equipped with a reverse phase C-18 column and a UV detector. Analyses for all the remaining compounds were performed on a Varian 4600 GC equipped with a Nickel-63 electron capture detector, a flame ionization detector and an SE54 30 m fused silica capillary column.

GC/MS quantification of EPA priority pollutants was accomplished by comparison with individual standards. All other quantifications were based on ion intensity relative to an internal standard and are accurate to within a factor of ten. DDT results were corrected for PCB interferences as previously reported (Lui-Hu 1979-1980) and all results by GC/EC, GC/FID, and HPLC were based on individual standards. Procedural blanks were analyzed with every set of sample extractions and all the sediment and tissue data was corrected for blanks and recoveries. The sediment results and volatile organics analysis were based on single samples. The remaining compounds are based on 3 to 5 replicates, depending on species.

Results

The first quarter effluent samples were analyzed by gas chromatography/mass spectrometry (GC/MS). This was an attempt to identify as many compounds as possible in the final effluent so that a subset of these could be chosen for subsequent analysis in sediments and tissues. From this analysis we were able to identify approximately one hundred compounds (Table 1).

The concentrations of identified compounds ranged from 0.003 ug/ℓ for o,p'-DDT to 2500 ug/ℓ for hexadecanoic acid. Of the one hundred compounds identified, 37 were acid extractable, 46 were base/neutral extractable, and 16 were volatile organics. Eleven of the compounds were present at concentrations greater than 100 ug/ℓ, while 65 were 10 ug/ℓ or greater. Approximately one-half of the base/neutral extractable and volatile organics were halogenated and only 3 of the acid extractable compounds were halogenated. Of the thirty seven compounds on the EPA Priority Pollutant List (U. S. Environmental Protection Agency 1977), 23 were

Table 1. List of compounds identified in L. A. County (JWPCP) Effluent collected November 1980.

Aldrin	2,5-dimethyl phenol	Ortho decyl-hydroxylamine
2,4-bis (1,1-dimethylethyl) phenol	Di-N-butyl phthalate	2,2'-oxybis propane
Bis (2-ethyl hexyl) phthalate	Dodecanoic acid	PCB 1242
Benzene	1-(2-ethoxy propoxy)-2-propanol	PCB 1254
Benzene methanol	1-ethyenyl, 2-methyl benzene	Penta chlorophenol
Benzoic acid	Ethyl benzene	Pentadecanoic acid
2-Butoxy ethanol	1-ethyl, 2-methyl benzene	Pentanoic acid
1-(2-butoxyethoxy) ethanol	3-ethyl phenol	Pentylcyclohexane
Butyl cyclohexane	4-fluoro biphenyl	Phenol
(1-butyl heptyl)-benzene	Heptachlor	Propanoic acid
Butyl octanol	Heptachlor epoxide	(1-propyl nonyl)-benzene
Caffeine	Hexachlorobenzene	2-propyl phenol
Chloroform	Hexadecane	Tetrachloroethylene
2,4'-DDD	Hexadecanoic acid	Tetrachlorophenol
4,4'-DDD	Hexanoic acid	Tetradecanoic acid
2,4'-DDE	Hexatricocane	Tetramethyl hexane
4,4'-DDE	4-hydroxy-4 methyl-2-pentanone	Tetramethyl pentane
2,4'-DDT	Methylcyclohexane	1,2,4-trichlorobenzene
4,4'-DDT	(1-methyl decyl)-benzene	1,3,5-trichlorobenzene
Decahydro 2-methyl naphthalene	Methyl heptanol	1,1,1-trichloroethane
1,2-dichlorobenzene	3-methyl octane	Trichloroethylene
1,4-dichlorobenzene	1-methyl-4(1-methyl) cyclohexene	2,4,5-trichlorophenol
1,2-dichloethane	1-methyl naphthalene	2,4,6-trichlorophenol
1,2-dichlorethene	2-methyl naphthalene	Triethyl silane
Dieldrin	4-methyl-2-pentanol	1,2,3-Trimethyl benzene
Diethyl phthalate	2-methyl-1-pentene	1,2,4-trimethyl benzene
1,2-dimethyl benzene	2-methyl phenol	Trimethyl dodecatrien-1-ol
1,4-dimethyl benzene	3-methyl phenol	Trimethyl 3-cyclohexene-1-methanol
Dimethyl disulfide	2-methyl propyles propanic acid	Trimethyl-1-hexane
3-(1,1-dimethyl ethyl)-phenol	Naphthalene	Trimethyl-1-nonene
1,2-dimethyl naphthalene	Nonadecanol	Toluene
2,3-dimethyl phenol	Octadecanoic acid	Undecane
3,4-dimethyl phenol	9-octadecenoic acid	Vinyl chloride
	Octanoic acid	

base/neutral extractable, 3 were acid extractable, and 11 were volatile organics. Eleven of these priority pollutants were above the quantification limit set by the EPA protocol and 7 of those were volatile organics.

The results of the analysis of surficial sediments (0-2 cm) and eight species of marine animals for the 27 selected compounds (percent dry/wet weight and percent lipid/wet weight), are presented in Table 2. The final breakdown of the 27 selected compounds was 10 base/neutral extractables, 4 acid extractables and 13 volatile organics covering a range of n-octanol/water partition coefficients (Log K_{OW}) of 1.48 to 6.19. In sediments and animals, DDE was present at the highest concentrations; Pacific sanddab liver contained the highest concentration of DDE at 122 mg/wet kg. Of the volatile organics, only toluene, benzene, trichloroethylene, and tetrachloroethylene were detectable in the tissues. The sediments were also scanned by GC/MS, but the presence of petroleum hydrocarbons prevented further identifications.

DISCUSSION

It would have been an overwhelming task to analyze the sediments and eight species of animals for all 99 compounds identified in the effluent or for all 120 Priority Pollutants. Therefore, we

Table 2. Results of the analysis of sediments (ug/dry kg) and benthic animals (ug/wet kg) from station PV 3.1 for the 27 selected compounds identified in L. A. County effluent.

Compound[a]	Sediment Conc	P. Sanddab Liver Conc	Halibut Liver Conc	Scorpion-fish Liver Conc	Dover Sole Liver Conc	Croaker Liver Conc	Crab Digestive GI Conc	Shrimp Muscle Conc	Inverts Whole Conc
pentachlorophenol	15	5	5	74	70	8	19	5	24
DDD (2)	558	2280	36	384	549	305	122	22	8
DDT (2)	191	1600	1870	422	168	20	344	9	5
DDE (2)	11700	122000	19200	20100	19000	21200	51900	294	383
Aroclor 1242	256	772	139	143	166	224	379	<2	13
phenol	10	NA	NA	NA	NA	NA	NA	NA	NA
Aroclor 1254	678	4920	2080	1140	615	1110	1200	18	19
methyl cyclohexane	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
tetrachlorophenol (3)	4	19	<1	<1	17	30	<1	<1	9
naphthalene	15	15	<20	<20	<20	42	<20	<20	<20
toluene	<1	<1	NA	<1	<1	25	NA	<1	<2
dichlorobenzene (2)	33	10	180	851	27	<1	575	403	59
trichlorophenol (2)	14	47	29	164	85	629	55	10	28
trichlorobenzene (2)	9	28	<1	15	7	<1	8	<1	<1
Heptachlor	7	40	8	5	3	1	5	<1	2
benzene	<1	<1	NA	16	52	15	NA	<1	8
ethylbenzene	<0.5	<0.3	NA	<0.3	<0.3	4	NA	<0.3	<0.3
hexachlorobenzene	5	29	2	5	6	4	5	<1	<1
dimethyl disulfide	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
dichloroethane	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
4-methyl-2-pentanol	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
tetrachloroethylene	<0.5	23	NA	29	19	11	NA	<0.3	8
chloroform	<0.5	<10	NA	<10	<10	<10	NA	<10	<10
1,1,1-trichloroethane	<0.5	7	NA	2	2	<0.3	NA	<0.3	4
trichloroethylene	<0.5	2	NA	6	4	2	NA	<0.3	7
1,2-dichloroethene	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
vinyl chloride	<0.5	<0.3	NA	<0.3	<0.3	<0.3	NA	<0.3	<0.3
Percent Dry/Wet Wt.	36.1	46.0	23.3	42.9	24.5	27.7	23.3	24.1	11.3
Percent Lipid/Wet Wt.	0.3	31.5	10.4	21.8	9.5	6.1	7.1	1.0	1.2

NA = Not Analyzed

[a]Numbers in parenthesis equals the number of isomers analyzed.

developed a method for selecting which compounds would be most important in terms of bioaccumulation and toxicity. We decided to call this the Toxic Bioaccumulation Factor (TBAF), which was computed for each compound by dividing the n-octanol/water partition coefficient (Hansch and Leo 1978) by the rat LD50 (National Institute for Occupational Safety and Health 1980). For example, the TBAF can be used to rank the Priority Pollutants by their relative importance. Table 3 presents twelve of the Priority Pollutants whose TBAF values are greater than 100. If all these compounds were discharged at the same rate, toxaphene would be of most importance because it has the highest potential to be acutely toxic. To make this principle applicable to L. A. County we multiplied the TBAF compound by its effluent concentration (Table 4). Of the compounds we selected, pentachlorophenol (PCP) has the highest TBAF value and is ranked number one on the list because it has the highest potential to bioaccumulate and to be acutely toxic. Of the top ten compounds, seven are halogenated (chlorinated). This indicates that halogens greatly increase the partition coefficient (and thus the potential for bioaccumulation), as well as adding to the toxicity.

The bioaccumulation of an organic compound is based on its ability to partition from the aqueous phase to the organic phase which, in tissues, are the lipids.

Table 3. The Toxic Bioaccumulation Factor (TBAF) ranking of several of the EPA's Priority Pollutants in order of relative importance.

Compound	Log[a] K_{ow}	LD50[b] mg/kg	TBAF[c]
Toxaphene	7.0	40	250000
DDT	6.19	113	13700
DDD	5.69	113	4330
Aroclor 1260	6.72	1315	3990
Parathion	3.81	2	3230
Pentachlorophenol	5.12	50	2640
Aroclor 1254	6.11	1295	995
DDE	5.69	880	557
Hexachlorobenzene	6.18	3500	432
Hexachlorobutadiene	4.33	90	238
Endrin	2.60	3	133
Aroclor 1248	6.11	11000	117

[a]Taken from Hansch and Leo, 1980.

[b]Taken from 1979 Registry of Toxic Effects of Chemical Substances.

[c]K_{ow}/LD50.

Table 4. Compounds identified in L. A. County (JWPCP) effluent and selected for analysis in sediment and tissues based on the TBAF.

Compound	Log[a] K_{ow}	LD50[b] mg/kg	TBAF[c]	Effluent conc ug/1	L. A. County TBAF[d]
pentachlorophenol	5.12	50	2640	2.3	6060
DDD (2)	5.69	113	4330	0.71	3080
DDT (2)	6.19	113	13700	0.087	1190
DDE (2)	5.69	880	557	0.19	106
Aroclor 1242	5.58	4250	89	0.94	84
phenol	1.48	414	0.1	980	71
Aroclor 1254	6.11	1295	995	0.052	52
methylcyclohexane	3.94	4000	2.2	20	44
tetrachlorophenol (3)	4.42	140	188	0.15	28
naphthalene	3.45	1780	1.6	15	24
toluene	2.73	5000	0.1	210	23
dichlorobenzene (2)	3.38	500	4.8	4.0	19
trichlorophenol (2)	3.69	820	6.0	2.4	14
trichlorobenzene (2)	4.08	756	16	0.81	13
Heptachlor	3.05	40	28	0.43	12
benzene	2.15	3800	0.1	220	8.2
ethylbenzene	3.15	3500	0.4	14	5.7
hexachlorobenzene	6.18	3500	432	0.013	5.6
dimethyl disulfide	1.77	805	0.1	45	3.3
dichloroethane	1.48	680	0.1	44	2.0
4-methyl-2-pentanol	1.90	800	0.1	8.0	1.4
tetrachloroethylene	2.60	8100	0.1	29	1.4
chloroform	1.98	800	0.1	8.0	1.0
1,1,1-trichloroethane	2.49	10300	0.1	31	0.9
trichloroethylene	2.29	4920	0.1	17	0.7
1,2-dichloroethane	1.98	770	0.1	5.2	0.6
vinyl chloride	1.52	500	0.1	6.2	0.4

[a]Taken from Hansch and Leo, 1980.

[b]Taken from 1979 Registry of Toxic Effects of Chemical Substances.

[c]K_{ow}/LD50

[d]K_{ow} X Effluent Concentration/LD50

Compounds that are water soluble are not taken up in the gut because they cannot pass through the lipoproteins in the gut lining; the more lipid-soluble a compound is, the easier it passes through the gut lining and enters the lipids of the body (Freed and Chiou 1981).

Laboratory studies have shown a linear relationship between the organic solubility of a compound and its potential for bioaccumulation. For example, Neely *et al.* (1974) obtained a straight-line relationship between the bioconcentration factor for several compounds in trout muscle (relative to concentrations in the laboratory exposure water) and the corresponding n-octanol/water partition coefficient. Also Karickhoff *et al.* (1979) demonstrated that the linear relationship of the sorption of compounds (aromatic and chlorinated hydrocarbons) onto river pond sediments and their water solubility could be estimated using the octanol/water partition coefficient. This coefficient has been widely used as a representative of this partitioning (Neely 1979; Maki 1979).

Most laboratory experiments examining uptake of organics have been based on exposing fish to compounds dissolved in the surrounding water. In our experiments, we measured the concentrations of the 27 selected compounds in the effluent, which represented our aqueous phase, and then in the sediments and tissues, which represented our organic phases. Based on these measurements we determined whether or not the effluent concentration multiplied by the n-octanol/water partition coefficient could be used to predict accumulation in the organic phases.

We found that we could not accurately predict relative accumulation in the organic phases using only effluent concentrations and partition coefficients for several reasons. First, the effluent discharged into the sea contains a high quantity of particulate substances to which organic compounds can attach. Since we did not separate the particulates from the effluent we did not have a true measurement of the concentration of contaminants which were actually dissolved in the aqueous phase. Second, sediment concentrations of some contaminants (e.g. total DDT) are unrelated to present day effluent concentrations because of historical discharges.

Despite these difficulties we found we could qualitatively predict which compounds would accumulate in the organic phases by identifying those compounds in effluent which had high partitions coefficients. This was demonstrated by comparing the ranking of the concentrations of compounds in the effluent to the concentrations in the sediments and the partition coefficient. The results of the Spearman's rank correlation are listed in Table 5. The effluent concentration was inversely correlated to the sediment concentrations ($r_S = -.553$) indicating that as the effluent concentration decreased the sediment concentration increased. This was predicted with the partition coefficient because the sediments are higher in organic content; the more organically soluble compounds should accumulate to a higher concentration while the effluent should contain higher levels of water soluble compounds since it is mostly water. When the effluent concentrations were compared to the partition coefficient (Log K_{OW}), they also had a significant inverse correlation ($r_S = -.806$), while the sediments and tissues correlated positively with the Log K_{OW}, suggesting that the Log K_{OW} closely represents the accumulation of these compounds and can be used to predict which compounds will accumulate there. Since the compounds with a high partition coefficient are partitioned into sediments, the sediments rather than the water must be the source of contaminants for the animals.

CONCLUSION

The complexity of effluents being discharged into the local coastal waters has long been a problem to scientists. Recent research has been directed towards identifying many of the chemical components (Eganhouse 1981); we identified only a small fraction of those in the effluent.

Table 5. Results of the non-parametric rank correlation test (r_s) indicating the relationship between the n-octanol/water partition coefficient (Log K_{ow}), effluent concentration, sediment concentration and tissue concentrations of the 27 selected compounds.

RANK B	RANK A	Log K_{ow}	Sediment	Pacific Sanddab Liver	Halibut* Liver	Scorpionfish Liver	Dover Sole Liver	White Croaker Liver	Crab* Digestive Gland	Shrimp Muscle	Whole Inverts
	Log K_{ow}	-	.737	.754	.666	.654	.687	.632	.703	.557	.467
	Effluent	-.806	-.553	-.691	-.565	-.520	-.535	-.403	-.600	-.502	-.333
	Sediment	-	-	.839	.948	.826	.849	.766	.949	.892	.747

$p < .01$ if $r_s \geq .491$

$p < .05$ if $r_s \geq .382$

*Volatile Organics were not analyzed in these samples, therefore we used the average value for all the other species for the statistical analysis.

We did, however, develop a method for predicting the relative importance of organic compounds that will aid in the consideration of which compounds have the highest potential for causing problems and should have the highest priority for research and monitoring. The Toxic Bioaccumulation Factor (TBAF) presented in Table 1 can be used to predict the relative ability of a compound to bioaccumulate and cause toxicity. This would then allow management decisions to be based on the relative importance to the specific area being considered.

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