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August 21, 1985

**FYI-0794-1181** AUG 28 REC'D  
B

Dr. Robert Brink  
Executive Secretary  
Toxic Substances Control Act  
Interagency Testing Committee  
401 M Street, S.W.  
Washington, D.C. 20460

Dear Dr. Brink:

Please refer to the information which I previously forwarded on the water insolubility of Colour Index Pigment Green 7 (Copper Phthalocyanine Green), on May 17, 1985. In that letter, I promised to forward hard analytical data, substantiating my claim for total insolubility of the above product in water.

I am now pleased to furnish additional information on laboratory studies performed under the supervision of Mrs. Maria daRocha, Sun Chemical's Manager of Analytical Services, and chairperson of the Analytical Committee of the Color Manufacturers' Association (DCMA).

As you will see, Copper Phthalocyanine Green (Colour Index Pigment Green) was found insoluble in water, at detection levels of less than 3 parts per million.

In the light of this finding, I would ask you to reconsider the need for further aquatic testing on this product, under the provisions of the Toxic Substances Control Act.

This letter is being copied to the Phthalocyanine Committee of DCMA. I look forward in the near future to receiving from you, the Interagency Committee's decision on this matter.

Sincerely,

SUN CHEMICAL CORPORATION  
Pigments Division

*Hugh M. Smith*

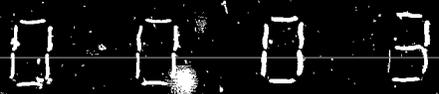
Dr. Hugh M. Smith  
Director of Research & Development

HMS/smk

cc: J.L. Robinson, Exec. Vice Pres., DCMA

Contains No CBI

RECEIVED  
SEP 11 AM 9:30



SUN CHEMICAL CORPORATION

<b>TO</b>	Dr. Hugh Smith	<b>FROM</b>	Maria DaRocha <i>Maria</i>
<b>LOCATION</b>	Cincinnati	<b>LOCATION</b>	Rosebank
<b>ANSWERING</b>		<b>DATE</b>	August 8, 1985
<b>SUBJECT</b>	<u>SOLUBILITY OF COPPER PHTHALOCYANINE GREEN IN WATER</u>		

Copper Phthalocyanine Green pigment contains approximately 6% copper. The solubility of phthalocyanine green in water was determined by measuring the water extractable copper content of the pigment by Atomic Absorption Spectrophotometry.

Prior to performing the extraction, the pigment was purified by dispersing in sulfuric acid, drowning in water, filtering and washing free of acid.

5 g of the purified pigment were slurried in 100 mls of de-ionized water, stirred at room temperature for 6½ hours, and allowed to stand for 36 hours at room temperature.

The amount of copper found was calculated to represent less than 3 ppm of phthalocyanine green.

MDR/rlp

HMS AUG 12 1985

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ETAD



Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry  
U.S. OPERATING COMMITTEE OF ETAD

August 20, 1985

AUG 22 REC'D  
RAB

Dr. Robert Brink  
Executive Secretary  
Interagency Testing Committee (TS-792)  
U.S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, D.C. 20460

REGISTERED

Re: Comments and information on C.I. Pigment Green 7  
ITC Intent to Designate, (50 FR 13418)  
Document Control No. OPTS-41017

Dear Dr. Brink,

I am pleased to submit to you, on behalf of ETAD, some information on C.I. Pigment Green 7.

Sincerely,

Eric A. Clarke  
Executive Secretary

EAC/bss  
Encl.

0005

Draft manuscript of a publication to be submitted to  
"Chemosphere". Not to be distributed before publication.

Aug 22 RECD

RHS

## THE LIMITS OF BIOACCUMULATION OF ORGANIC PIGMENTS IN FISH<sup>1</sup>

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Manufacturing Industry (ETAD), P.O. Box, CH-4005 Basle 5,  
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Basle, Switzerland

### ABSTRACT

A reliable experimental determination of  $P_{ow}$  of organic pigments is met with serious difficulties due to the extremely low water solubilities of these compounds. Therefore the  $P_{ow}$ -values and the water solubilities were calculated for eleven typical organic pigments and some disperse dyes as well. The  $P_{ow}$ -values of the pigments were very high predicting bioaccumulation factors (BF) several orders of magnitude above 1000. Based on recent studies confirming that n-octanol simulates lipids in their solubilizing effect on organic chemicals, the solubilities of these organic pigments in n-octanol were measured in order to estimate their potential for lipid storage. The very low solubility values indicate that in spite of the very high predicted BF such pigments cannot build-up concentrations in lipids (hence in fish) which could be of concern considering their generally low toxicity and the extremely small amounts entering the environment. Therefore there should be no need to perform a fish bioaccumulation test for assessing the bioaccumulation potential of such compounds provided they show comparable solubility characteristics as the pigments investigated in this study.

<sup>1</sup>Based on a paper presented at the International Symposium on "Bioavailability of Environmental Chemicals" held in Schmallenberg-Grafschaft, FRG, on September 12-14, 1984.

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## INTRODUCTION

Recently it has been shown that the partition coefficient in octanol/water ( $P_{ow}$ ) is a useful indicator of the bioaccumulation tendency (BF) of organic ionic and non-ionic dyestuffs (1). If the  $P_{ow}$  is  $< 1000$ , it can confidently be predicted that the bioaccumulation factor (BF) in fish will be  $< 100$ . So far results of fish bioaccumulation tests with over 75 dyestuffs have confirmed this empirical rule.

Neely et al. (2), and several authors since, for a review see (3), have described linear correlations between  $\log BF$  and  $\log P_{ow}$ , but mainly for the highly lipophilic halogenated hydrocarbons. On the basis of any of the established correlation equations bioaccumulation would have been predicted in the case of the relatively lipophilic disperse dyes ( $\log P_{ow} > 3$ ) and of some organic pigments (calculated  $\log P_{ow}$  up to 9). However, the experimental studies showed that the 20 disperse dyes and pigments investigated did not bioaccumulate in fish. For disperse dyes it was hypothesized that this behaviour may be due to relatively large size (molecular weights ca. 450-550) making transport across membranes difficult (1).

Other factors which might become critical and which must be considered when estimating the bioaccumulation potential of ionic, and non-ionic chemicals have been discussed by Esser and Moser (4), and in the OECD Guidelines (5). The pigments did not seem to accumulate despite their generally very high calculated  $\log P_{ow}$ -values which would predict bioaccumulation factors several orders of magnitude above  $10^2$ . Due to the extremely low water solubilities of the pigments it was not possible to determine the real BF. In order to work in a practical and experimentally accessible concentration range the pigments had to be finely dispersed in the water used for the fish accumulation test. No accumulation in the fish as compared with the amount of pigments dispersed in the test-water was observed (1).

To investigate this phenomenon further the measurements of the solubilities of a number of organic pigments and disperse dyes in n-octanol for comparison with the solubilities of strongly bioaccumulating compounds, and the calculation of the  $P_{ow}$  and the water solubilities seemed desirable.

It was shown recently by Chiou (6), that octanol simulates fats in respect to their partition behaviour very well, and it can be assumed that this is also the case for its solubilizing effects for organic chemicals. Although Dobbs and Williams (7) have found no direct correlation between  $\log BF$  and  $\log$  fat solubility for ten chemicals, the solubility in octanol is an indicator of the maximum fat storage of a chemical, it is as will be shown in this paper.

## METHODS AND EXPERIMENTAL

The experiments reported thereafter are the results of a collaborative effort of analytical Laboratories of different ETAD Member Companies and were conducted according to similar prescriptions.

### Chemicals

Disperse dyes and pigments used in the measurements were of the purest qualities available in the different laboratories. They were generally recrystallized several times or purified by solvent extraction. All other chemicals were of the highest available commercial quality and were used for analysis without further purification. n-Octanol was of analytical quality from Fluka AG (or Riedel de Haen), and was further distilled over a wire mesh column. For solubility measurements dry n-octanol was used, while for measurements of the partition coefficient, the n-octanol was carefully equilibrated with doubly quartz distilled water before use.

### Determination of octanol/water partition coefficients

n-Octanol/water partition coefficients,  $\log P_{OW}$ , of the compounds were determined by the flask-shaking method according to established procedures (e.g. OECD Method 107 (5)), if they were in an experimentally accessible range and if the compounds were soluble enough in both phases.

In addition, all partition coefficients were calculated, using either the schemes developed by Rekker (8) or of Hansch and Leo (9). The latter was available as a computer program, CLOGP 3.2 (10). The calculated log P's given in Tables 1 and 2 are mostly the CLOGP 3-values, corrected, if necessary, by hand for intramolecular H-bonds. For some compounds the results obtained by the Rekker scheme, using some of our own fragment constants were considered more trustworthy.

### Determination of the solubility in octanol

Somewhat different procedures were used for the solubility measurements of the disperse dyes and the less soluble pigments.

### Disperse dyes and reference compounds

5 g of the highly purified dyestuff was weighed into a ground glass necked Erlenmeyer flask and suspended in 75 ml of pure n-octanol. The suspension was stirred for 8 hours in a thermostat at 20°C, keeping the flask stoppered, and then filtered through a G4 fritted filter. Aliquots of the filtrate were diluted with acetone and their absorbance

measured spectrophotometrically at the peak wave length. In a duplicate experiment the stirring time was 16 hours. Constancy of the values was taken as confirmation that equilibrium conditions had been reached after 8 hours. The undissolved parts of the dye-stuff were sucked dry on the filter, again suspended in 75 ml of n-octanol and treated as described. This procedure was repeated four more times to eliminate adverse influences of possible impurities. The still undissolved dyestuff powder was vacuum-dried at 40°C. With this material reference solutions were prepared.

#### Pigments

The pigment samples were pretreated by extracting for 2 hours with n-octanol in a Soxhlet apparatus, then vacuum-dried at 100°C. If necessary the extraction was repeated. 5 g of pretreated pigment was then suspended in 75 ml of pure n-octanol for 8 hours. The suspension was stirred at room temperature. In a parallel experiment the sample was stirred for 16 hours. After this period the suspensions were left without stirring for 2 to 8 hours and then filtered through a G5 frit. To ease the filtration the suspensions were first centrifuged. The filtered solutions were kept for measurement. The combined filter and centrifuge residues were suspended again in 75 ml of n-octanol and the whole procedure repeated until the measured concentration in the solution remained sufficiently constant. The spectrophotometric concentration determinations were performed using standard solutions in n-octanol or where necessary in n-octanol-acetone. Since dissolution rates for barely soluble substances are low, it was tested with pigment XII whether saturation was actually reached under the experimental conditions given above. In one experiment the stirring time was increased to 72 hours. In a parallel experiment the pigment was stirred for 2 hours at 50°C and then for 16 hours at 20°C. Both experiments gave essentially the same results (17.3 and 17.6 mg/l) as the standard method (17.0 mg/l) which were within the standard deviation.

#### Calculation of solubility in water

The water solubilities,  $C_s^W$  (Concentration at saturation in water), of most disperse dyes and pigments were not known and judged to be too small for a trustworthy experimental determination. In order to get an idea about the magnitude of  $C_s^W$  nevertheless, the solubilities were calculated from four different semiempirical relations (11, 12, 13, 14). All four equations take into account the phase transition from a crystalline solid to supercooled liquid and are based on empirical correlations between measured water solubilities and partition coefficients. Most of these correlations, however, have been established with rather simple compounds such as aromatics, chlorinated hydrocarbons and PCBs. As can be seen from the values at the bottom of Table 1, the predictions for substances of this type are quite satisfactory (in these cases the range of  $C_s^W$  is given, as obtained from all four equations). For the disperse dyes and pigments a larger uncertainty of the predicted solubility must be expected because a) the four regression equations have to be extrapolated further in respect to  $\log P_{ow}$  and melting

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point and because b) the applicability of the regression equations derived for simple compounds to such complex chemical structures will be limited. Particularly the equation derived by Banerjee et al. (11) seems to be inadequate for compounds with melting points above 150°C and was not used in these cases. We expect from experience that the results for  $C_S^W$  will be trustworthy to within say  $\pm$  one order of magnitude. We attempted to calculate also the octanol solubilities,  $C_S^O$  from  $P_{OW}$  and the calculated water solubilities according to a scheme discussed in chapter 3.3. of the book of Lyman et al. (15) from  $P_{OW} = C_S^O / C_S^W$ . However, since this approximation is valid only if both  $C_S^O$  and  $C_S^W$  are small - and this is generally not the case for  $C_S^O$  - and because uncertainties from  $C_S^W$  and  $P_{OW}$  are greatly amplified by such a simple procedure, large errors in  $C_S^O$  have to be envisaged. Thus they are not given in Tables 1 and 2.

## RESULTS AND DISCUSSION

A comparison of the log octanol solubilities of all seven reference compounds in Table 1 with the log fat solubilities reported by Dobbs and Williams (7) shows that, with the exception of  $\gamma$ -hexachlorocyclohexane, where a difference of one log unit is found, the differences are between 0.2 and 0.7 log units with an average difference of 0.4. This good agreement indicates that the solubility in octanol reflects quite well the solubility of chemicals in natural lipids. This finding forms a parallel to the work of Chiu (6) who found a close correlation between partition coefficients in triolein/water ( $P_{TW}$ ) and  $P_{OW}$  suggesting that  $P_{OW}$  is also an excellent predictor for  $P_{TW}$  i.e. for lipid/water partition coefficients and bioconcentration factors.

It seems reasonable to assume, that the good correspondence between octanol and lipid solubility is also valid in the range of lower solubility so that the measured solubilities of the pigments and dyes in octanol (Table 1 and 2) will be reasonably close to their solubility in lipids - and lipids, as shown clearly in a recent paper by Geyer et al. (16) is certainly the main, if not the only storage site for lipophilic chemicals in fish. Thus, even if the partition coefficients of most pigments (see Table 2) would indicate bioaccumulation factors up to several orders of magnitude above  $10^2$ , the uptake of these chemicals will be limited at a ceiling given by the value of their lipid solubility.

A simple calculation may serve to illustrate this fact:

If pigment XV (see Table 2) is taken as a typical example, its (calculated) water solubility of between  $2 \cdot 10^{-6}$  and  $10^{-5}$  ppm and its  $\log P_{OW} = 8.10$  would theoretically predict a lipid concentration of between 250 and 1250 ppm. However, its measured octanol (= lipid) solubility is only 0.46 ppm, i.e. between 550 and 2700 times less. Based on a typical lipid content of fish of 5% (15), the pigment content of the fish on a wet weight basis would be at maximum  $0.05 \times 0.46 = 0.023$  ppm which, of course, is almost at the practical limit of analytical de-

tectability and in view of the very low order of toxicity of organic pigments (17, 18, 19, 20) certainly beyond any potential hazard. In addition, this figure will probably represent an upper limit which will not be reached under practical conditions because of the molecular weight effect as also suggested for disperse dyes, i.e. a substantial inhibition of permeation through membranes and other biological materials due to the large molecular size of these organic pigments.

Thus, the assessment of the bioaccumulation of very sparingly soluble chemicals such as the pigments, must be based on a calculation as shown above rather than a log BF vs. log  $P_{ow}$  correlation equation which does not take into account the solubility of the chemical in lipids or in water and thus may lead to completely wrong conclusions.

For disperse dyes with substantially higher octanol (lipid) solubilities, the same arguments lead to the conclusion, that the bioaccumulation (also up to the maximum lipid solubility) may reach much higher levels which may thus warrant the execution of a fish accumulation test - if it is indicated by the magnitudes of log  $P_{ow}$  (i.e. > 3) and  $C_s^W$  (i.e. < 2 g/l).

#### CONCLUSIONS

Colorants (dyestuffs and organic pigments) are not readily biodegradable (21) as defined by the OECD Guidelines for Testing of Chemicals (5). Existing regulations require in such cases the performance of fish bioaccumulation tests (1).

The numerical value of the n-octanol/water partition coefficient  $P_{ow}$  has become an important factor to be considered in determining whether to conduct the expensive fish bioaccumulation studies.

It has been shown (1) that the  $P_{ow}$  provides a useful indicator of bioaccumulation tendency also for ionic and non-ionic dyestuffs. If the  $P_{ow}$  is less than 1000 it can be confidently predicted that the BF in fish will be less than 100. Consequently there is no need to conduct a fish bioaccumulation study with such substances.

The experimental determination, however, of the  $P_{ow}$  of the practically water-insoluble pigments may be extremely difficult. In such cases it is suggested to calculate the  $P_{ow}$ . As shown in this study the calculated  $P_{ow}$ -values of the investigated organic pigments were up to several orders of magnitude above the critical value of 1000 with the exception of pigment salts represented by compound XVII, Figure 2. The high  $P_{ow}$ -values would suggest strong bioaccumulation tendencies. But no accumulation in the fish as compared with the amount of pigments (e.g. pigments XXII and XXIII) dispersed in the test-water was observed (1) for any of the pigments.

The reason for this apparent inconsistency is the very limited fat (lipid) storage potential of these pigments indicated by their very low solubilities in n-octanol. As a consequence the procedure to follow in assessing the bioaccumulation potential of pigments would be

- measurement or calculation of the  $P_{OW}$  and  $C_S^W$ , and
- determination of the solubility in n-octanol, specially for those with  $P_{OW} > 10^3$ .

As shown above the upper limit of possible concentrations in fish can then be determined very easily from n-octanol solubility values and the percentage of lipid content in fish. In such cases there is definitely no need to perform a rather complicated fish accumulation test and to unnecessarily sacrifice test animals.

Based on this value and taking into account other parameters including water solubility, amounts introduced in the aquatic environment, and ecotoxicological and toxicological properties, it can be assessed whether any potential hazard may exist.

#### ACKNOWLEDGEMENT

We thank the Members of the ETAD Analytical Subcommittee, Dr. E. Bankmann, Dr. R. Hotz, Mr. W.G. Sharples, Dr. H. Steuerle, Dr. M. Störi, and Dr. H. Weis, for the determination of the solubilities of colorants mentioned in this paper.

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**Table 1** Partition Coefficients, Water- and n-Octanol-Solubilities, and Bioaccumulation Factors of Disperse Dyes and some Reference Compounds

Compound	MW	Melting Point °C	log P <sub>ow</sub>		Solubility in water (mg/l) 20°C		log BF in fish <sup>k</sup>
			exp. b	calc. c	exp. b	calc. e	
I	546.38	175	2.5	3.32	ND	20-100	1.0
II	333.00	225	3.4	2.19	ND	0.16-2.5	0.48
III	363.40	225	> 4.0	3.50	< 0.01	0.5-6.5	< 0.70
IV	368.19	219	> 4.0	4.40	< 0.01	0.15-0.6	0.70
V	448.53	143	4.0	4.65	ND	0.8-4.7	< 0.70
VI	423.30	184	> 2.0	4.41	ND	0.07-0.78	< 0
VII	426.90	170	4.0	3.54	ND	0.37-2.8	1.76
VIII	528.90	117	4.1	5.35	ND	2.8-9.2	0.70
IX	373.83	145	3.9	3.03	ND	1.0-5.3	0.30
X	411.54	173	4.5	5.13	ND	0.02-0.2	0.90
Atrazine	215.72	177	2.63	2.82	33 <sup>a</sup>	7-11	0.48 <sup>1</sup>
Coronene	300.36	440	N.D.	7.04		3.10-9.4, 10-6	14'400 <sup>a</sup>
DDT	354.48	109	4.06-6.36 <sup>f</sup>	6.91	0.012-0.04	0.01-0.06	28'600-31'800 <sup>a</sup>
Hexachlorocyclohexane	290.82	160	3.80 <sup>f</sup>	3.75	91 (for γ)	0.6-3.8	ca. 15'000
Hexachlorobenzene	284.76	230	5.47 <sup>g</sup>	6.42	0.005h-c.059	0.006-0.02	4'350
p-Dichlorobenzene	147.00	53	3.38 <sup>f</sup>	3.57	319	60-70	260'000 <sup>a</sup>
1,2,4-Trichlorobenzene	181.45	17	4.12 <sup>f</sup>	4.28	469	29-45	miscible

<sup>a</sup> Own measurement (P.M.)

<sup>b</sup> Measurements by ETAD Member Companies

<sup>c</sup> Calculated by Pomona College Med. Chem. Project computer program CLOGP 3.2 or by Rekker's scheme

<sup>d</sup> The log BF varies in a wide range depending on the variety of fish

<sup>e</sup> Range of solubilities calculated from 3 or 4 regression equations as explained in the text. If available or considered trustworthy, the measured log P was used, otherwise the calculated log P

<sup>f</sup> From log P-table of Pomona Med. Chem. Project (10)

<sup>g</sup> see Ref. 14

<sup>h</sup> Yalkowsky, S.H.; Orr, R.J.; Valvani, S.C., Ind. Eng. Chem. Fundam. 18, 351 (1979)

<sup>i</sup> see Ref. 3

<sup>k</sup> Fish bioaccumulation data of disperse dyestuffs were obtained by ETAD Member Companies according to the procedure specified by the Japanese authorities under the Chemical Substances Control Law Act. The BF values were expressed on the basis of wet body weight of the fishes. For references see (1). Procedures for de-terminations of bioaccumulation in various fish species for some specific dyestuffs are described in ETAD Methods Nos. 203-209, 211, 213, 216, and are available at the ETAD Secretariat

<sup>l</sup> see Ref. 16

**Table 2** Partition Coefficients, Water- and n-Octanol-Solubilities, and Bioaccumulation Factors of Pigments.

Compound	MW	Melting Point °C	log P <sub>ow</sub> calc.	Solubility in water (mg/l) 20°C calc.	Solubility in n-octanol (mg/l) exp. <sup>a</sup> 20°C	log BF exp.
XI	340.33	256	3.82	0.2 - 2	9.4	ND <sup>b</sup>
XII	307.29	276	5.35	0.002 - 0.02	17	ND
XIII	486.76	290	8.38	2.10 <sup>-6</sup> - 10 <sup>-5</sup>	7.8	ND
XIV	629.50	320	6.80	5.10 <sup>-5</sup> - 5.10 <sup>-4</sup>	0.5	ND
XV	726.44	320	8.10	2.10 <sup>-6</sup> - 10 <sup>-5</sup>	0.46	ND
XVI	818.50	400	7.10	3.10 <sup>-6</sup> - 2.10 <sup>-5</sup>	< 0.5	ND
XVII	426.45	360	0.73	1 - 60	< 1.5	ND
XVIII	439.78	330	3.40	0.1 - 5	3.5	ND
XIX	312.32	> 400	4.30	3.10 <sup>-4</sup> - 2.10 <sup>-3</sup>	1.7	ND
XX	1092-1127	480	17.40	7.10 <sup>-18</sup> - 2.10 <sup>-16</sup>	0.07	ND
XXI	576.05	480	6.60	8.10 <sup>-7</sup> - 3.10 <sup>-4</sup>	< 0.05	ND
XXII	760.10	350	10.50	3.10 <sup>-9</sup> - 2.10 <sup>-8</sup>	< 1	0.48 <sup>a</sup>
XXIII	674.36	300	10.10	3.10 <sup>-8</sup> - 10 <sup>-7</sup>	< 0.1	0.70 <sup>a</sup>

<sup>a</sup> Measurements by ETAD Member Companies

<sup>b</sup> Not determined

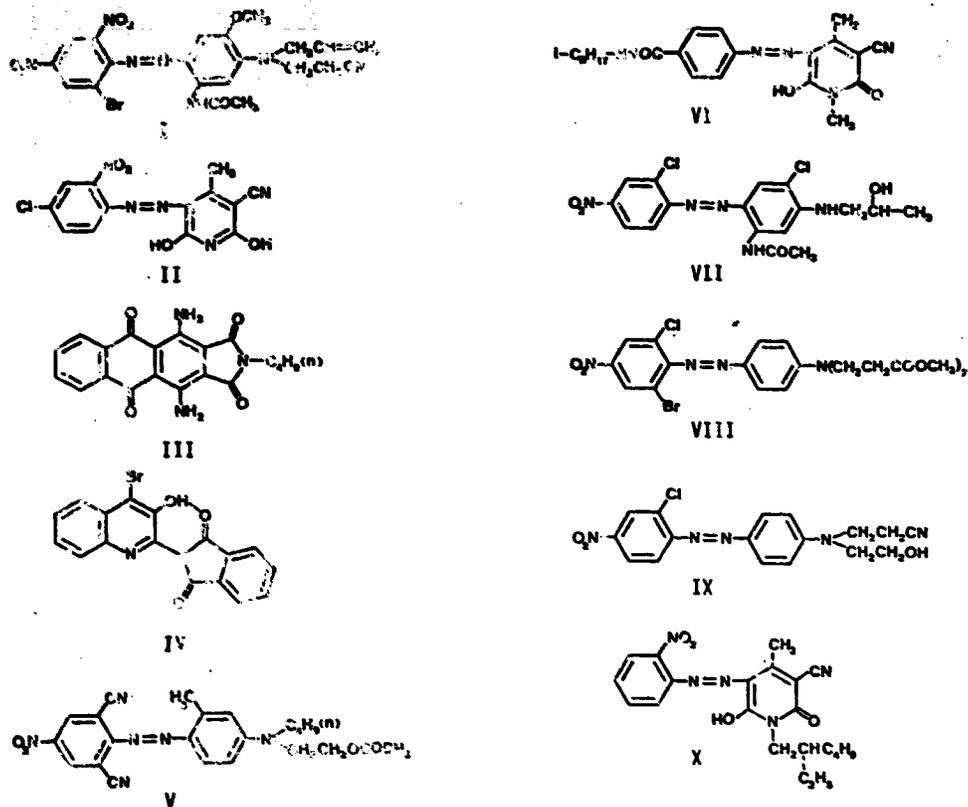


Figure 1 Disperse Dyestuffs investigated. Values compiled in Table 1

I, V, VII, VIII, IX	Nitrazobenzene-type
II, VI, X	Phenylazopyridone-type
III	Anthraquinone-type
IV	Hydroxyquinoline-type

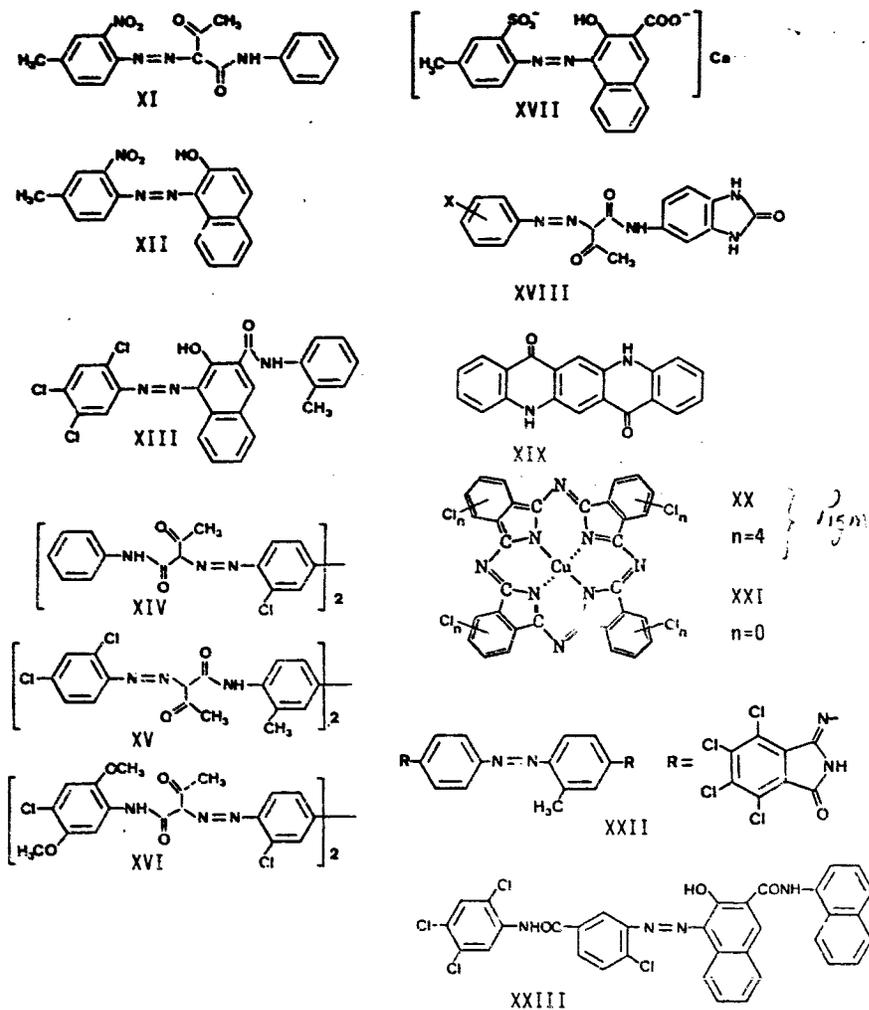


Figure 2 Organic Pigments investigated. Values compiled in Table 2.

XII	Phenylazonaphthol-type
XI, XVII	Monoazo-acetoacetylde-type
XIV, XV, XVI	Disazo-acetoacetylde-type
XIII, XXIII	Phenylazo-2-hydroxy-naphthoic acid-type
XVII	Salts of Phenylazo-2-hydroxy-naphthoic acids
XXII	Tetrachloroisindolinone-type
XX, XXI	Phthalocyanines
XIX	Quinacridones

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USE OF THE PARTITION COEFFICIENT AS AN INDICATOR  
OF BIOACCUMULATION TENDENCY OF DYE STUFFS IN FISH

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ABSTRACT

*Dyestuffs generally do not readily undergo aerobic biodegradation during sewage treatment processes and for new products an assessment of their bioaccumulation in fish is a requirement under certain environmental chemicals legislation. The results presented in this paper show that the partition coefficient in n-octanol/water is a useful indicator of the bioaccumulation tendency of dyestuffs, thus supporting its use as a screening test for bioaccumulation as proposed by the OECD Chemicals Testing Programs.*

INTRODUCTION

Synthetic organic dyestuffs are not readily biodegradable during effluent treatment processes and, if not removed by adsorption processes, may enter the aqueous environment. Although the amounts involved are relatively small, and are being reduced by improved application technology and effluent treatment, an assessment of their possible environmental impact is necessary and this may involve consideration of their bioaccumulation tendency.

Under the Japanese "Chemical Substances Control Act (1974)" new products which are not readily biodegradable must pass a bioaccumulation test in fish before they can be introduced to the market (1). The fish is an appropriate test animal because of its position in the food chain, and also because, especially in some communities, it forms an important constituent of the human diet.

However, since fish accumulation testing is expensive and time consuming, considerable interest was aroused by the evidence that the partition coefficient (P) in n-octanol/water provides a useful indication of bioaccumulation tendency (2,3) and indeed also of soil adsorption (4) and bio-

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magnification (5). Subsequently, the relevance of partition coefficient has been acknowledged in the new product notification schemes (6) being enacted in the U.S.A. under the Toxic Substances Control Act (1976) and in the EEC (7). Within the framework of the OECD Chemicals Testing Programme (8) it is proposed to use P as a criterion for screening products for bioaccumulation tendency.

This paper examines the relevance of P for predicting bioaccumulation tendency in fish in the case of synthetic organic dyestuffs, which represent a wide variety of fairly complex chemical structures possessing widely differing physico-chemical properties.

#### EXPERIMENTAL

##### Measurement of fish bioaccumulation

All fish bioaccumulation data were obtained by the experimental procedures specified by the Japanese authorities under the Chemical Substances Control Law. The experimental details are not included here, but have been described extensively elsewhere (1,9). The data included are based on the pooled results obtained by the ETAD member companies<sup>1</sup> in seeking to register new dyestuffs in Japan.

The bioaccumulation factors (BF) reported in Table 1 were obtained from the registration reports of the ETAD member companies to the Japanese Ministry of International Trade and Industry (MITI), which detail the BF's at two concentration levels and various time periods.

##### Partition coefficients (P) between n-octanol and water

###### Experimental determination of partition coefficients

Most partition coefficients between n-octanol and aqueous phases were determined by the shake flask method using a procedure similar to that proposed by the OECD Expert Group on Physical Chemistry (10). In general, the aqueous phase consisted of doubly distilled water, but buffers and salt solutions were used when necessary (11). In all cases careful mutual presaturation of both phases by equilibrating large stock volumes was carried out. After partitioning, the concentration in one or, if possible, in both phases was determined spectrophotometrically.

The following companies are members of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD): ACNA S.p.A., Amar Dye-Chem Ltd, BASF AG, Bayer AG, C.A. Venetolana de Pigmentos, Ciba-Geigy AG, Daito Chemical Ind. Co. Ltd, Hodoqaya Chemical Co. Ltd, Hoechst AG, ICI Ltd, Indian Dyestuff Industries Ltd, Kôge Chemical Works Ltd, Mitsubishi Chemical Industries Ltd, Mitsui Toatsu Chemicals Inc., Nippon Kayaku Co. Ltd, Produits Chimiques Ugine Kuhlmann, Rohner AG, Sandoz AG, Sumitomo Chemical Co. Ltd, Yorkshire Chemicals Ltd.

Impurities, which may be present in the commercial dyestuffs, may interfere with the partition determinations. In such circumstances a convenient procedure is to conduct multiple extractions (rejecting the less colored layer and replacing it with fresh equilibrated phase) until a stable P value is obtained. In this context the use of "reverse-phase" liquid chromatography shows considerable promise.

#### Calculation of partition coefficients

Partition coefficients were calculated by the fragment methods of Hansch and Leo (12) or of Reker (13,14) and the  $\pi$ -method of Hansch (15). Larger fragments like heterocyclic rings or fused rings were taken or derived from the log P-tables of Hansch and Leo (16). For the negatively charged  $\text{SO}_3^-$ -groups in the acid or reactive dyes the  $\pi$ -value of -4.76 was taken from Hansch (15), while for a positive charge on either an aliphatic or aromatic quaternary nitrogen, a charge contribution of -5.0 was added to the sum of fragment values. This value seems to account best for this type of charge effect, as judged from our experience.

### RESULTS AND DISCUSSION

#### Partition coefficients

Table 1 gives the bioaccumulation factors, and the calculated and experimental P values for 75 dyestuffs, together with some indication of their structural features. Part A includes those dyes for which experimental log P values were determined and checked by calculation. These lists include representatives from nearly all major groups of dyestuffs and as judged from the log  $P_{\text{calc}}$ , the lipophilicities of these products range from extremely hydrophobic to extremely hydrophilic, i.e. the partition coefficients lie within a range of  $10^{20}$ .

Fig. 1 compares the measured and calculated log P values given in Table 1 (Part A).

In one case, compound no. 12, the log P could not be determined experimentally. This triaryl-methane compound is extremely lipophilic ( $\log P_{\text{calc}} = 11$ ) and its water solubility has been estimated to be less than  $10^{-12}$  g/l (as calculated from the log  $P_{\text{calc}}$  and the measured n-octanol solubility). Because of this extremely low water solubility, its concentration in the aqueous phase could not be measured.

Some comments on the correlation between the measured and calculated log P values of Fig. 1 are warranted, as this shows some interesting features.

1. In the region above  $\log P = -2$  deviations of up to ca 1.5 log units occur. Such deviations are expected and are explicable in terms of impurities, association phenomena, and solubility and analytical problems which influence the precision of the experimental determination and

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TABLE 1 (Part A) Fish Bioaccumulation Factors, Experimental and Calculated Partition Coefficients (n-Octanol/Aqueous Phase) for Dyestuffs<sup>1)</sup>

No	Structural type	Charge type	Fish Bioaccumulation factor log BF	Partition coefficient log P	
				expt	calc
1	Disperse, monoazo		0.0	>2.0	4.4
2	Disperse, monoazo		1.0	2.6	3.4
3	Disperse, monoazo		0.7	4.1	3.6
4	Disperse, monoazo		0.9	4.5	4.6
5	Disperse, monoazo		0.7	4.5	5.5
6	Disperse, monoazo		<1.2	5.1	3.9
7	Disperse, monoazo		<0.7	4.0	3.5
8	Disperse, monoazo		0.48	3.4	2.2
9	Disperse, monoazo		1.70	~4.0	3.3
10	Disperse, anthraquinone		<0.7	>4.0	4.3
11	Disperse, fused heterocycle		0.6	3.2	3.5
12	Triarylmethane		1.2	>>4.0 <sup>2)</sup>	11.0
13	Disazo, Cr-Complex (1:2)		1.74	1.5 to 2.4	1.7 <sup>3)</sup>
14	Disazo, Cr-Complex (1:2)		1.11	-0.3 to 0.3	1.6 <sup>3)</sup>
15	Disazo, Co-Complex (1:2)		0.50	1.5 to 2.1	1.6 <sup>3)</sup>
16	Acid, monoazo	-	<0.6	0.10	-3.3
17	Acid, monoazo	-	0.56	0.21	0.0
18	Reactive, monoazo	2 -	0.30	-2.05	-5.2
19	Reactive, monoazo	2 -	0.08	-2.10	-3.5
20	Reactive, monoazo	3 -	<-0.7	-2.40	-10.0
21	Reactive, monoazo	3 -	-0.09	-1.74	-8.0
22	Reactive, monoazo	3 -	0.0	-2.90	-10.0
23	Reactive, monoazo	3 -	0.0	-2.3	-12.0
24	Reactive, monoazo	3 -	- <sup>4)</sup>	<-2.0	-10.0
25	Reactive, anthraquinone	2 -	-0.12	-1.50	-2.6
26	Reactive, formazone Cu-complex	3 -	0.0	-2.2	-7.0
27	Reactive, disazo	4 -	0.0	-2.2	-13.0
28	Basic, methine	+	<0.8	-0.2	1.0
29	Basic, methine	+	0.35	0.40	0.40
30	Basic, methine	+	-0.2	-0.52	-1.0
31	Basic, disazo	+	-0.3	-0.66	-1.0
32	Basic, oxazine	+	0.2	0.8	-3.4

Footnotes to Table 1 (Part A)

- 1) This table contains all dyes, for which an experimental determination of the partition coefficient has been carried out or attempted. Experiments have been performed in different laboratories, most of them by one of the authors (P.M.).
- 2) Experimental determination not possible due to exceedingly low water solubility (<0.1 mg/l).
- 3) Calculated on the basis of the uncharged ligand. The whole complex carries no net charge.
- 4) Not determined, product accepted by analogy.

TABLE 1 (Part B) Fish Bioaccumulation Factors and Calculated Partition Coefficients (n-Octanol/Aqueous Phase) for Dyestuffs<sup>1)</sup>

No	Structural type	Charge type	Fish Bioaccumulation Factor log BF	Calculated Partition Coefficient log P <sub>calc</sub>
33	Disperse, monoazo		0.6	3.0
34	Disperse, monoazo		n.d. 2)	-3.0
35	Disperse, monoazo		1.3	2.2
36	Disperse, monoazo		1.3	1.8
37	Disperse, monoazo		1.3	1.6
38	Disperse, monoazo		0.8	2.9
39	Disperse, monoazo		0.3	4.6
40	Disperse, monoazo		0.6	2.1
41	Disperse, monoazo		0.0	2.9
42	Disperse, anthraquinone		0.7	2.1
43	Disperse, anthraquinone		0.7	2.6
44	Disperse, quinoline		0.7	3.3
45	Pigment, monoazo		0.7	9.5
46	Pigment, monoazo		0.5	9.3
47	Phthalimidine		0.6	5.2
48	Acid, monoazo	-	<0.7	-0.1
49	Acid, monoazo	-	n.d. 2)	-2.1
50	Acid, monoazo	3 -	<0	<0
51	Acid, disazo	-	0.7	0.01
52	Reactive, monoazo	3 -	<0.5	<0
53	Reactive, monoazo	2 -	<0.7	<0
54	Reactive, monoazo	3 -	-0.4	<0
55	Reactive, monoazo	4 -	<0.1	<0
56	Reactive, monoazo	-	n.d. 2)	-1.8
57	Reactive, monoazo	3 -	<0	<0
58	Reactive, monoazo	3 -	<0	<0
59	Reactive, monoazo	-	0.5	-0.4
60	Reactive, monoazo	2 -	0.6	<0
61	Reactive, monoazo, Cu-complex	3 -	<0.3	<0
62	Reactive, monoazo, Cr-complex	-	0	-2.2
63	Reactive, monoazo, Cu-complex	3 -	n.d. 2)	<0
64	Reactive, triazine, Cu-complex	5 -	0.7	<0
65	Reactive, disazo	5 -	-0.2	<0
66	Reactive, disazo	6 -	<0.1	<0
67	Reactive, disazo	5 -	<-0.4	<0
68	Reactive, disazo, stilbene	4 -	n.d. 2)	<0
69	Reactive, phenazine	3 -	<0.4	<0
70	Reactive, phenoxazine	3 -	0.4	<0
71	Reactive, phenoxazine	8 -	0.9	<0
72	Reactive, Cu phthalocyanine	3 -	1.2	<0
73	Direct, disazo	3 -	0.2	<0
74	Basic, methine	+	0.7	1.8
75	Basic, oxazine	+	0.7	1.9

Footnotes to Table 1 (Part B)

- 1) This table contains those dyes for which no experimental determinations of the partition coefficients were available.  
 2) No substance detected in the test fish.

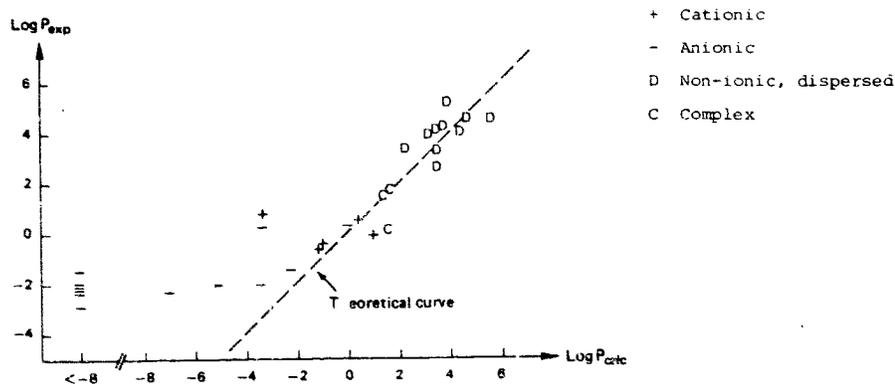
on the other hand the difficulties in choosing the correct lipophilic fragment values and interaction terms. This leads to uncertainties in the calculation of the log P value, which, even when carried out by an experienced scientist, can amount to an error of up to a factor of 100 in the P value.

2. In the region below  $\log P = -2$  an interesting phenomenon is observed. Even for the most hydrophilic dyes, carrying two or more charged groups, the experimentally measured log P is only between  $-2$  and  $-3$ .

This limiting value is probably set by the relatively large solubility of water in octanol (4.45 g/100 ml at 25°C) and the concomitant presence of a minimum concentration of hydrated dye-ion pairs in the organic phase. This happens even when substance concentrations for measurements have been chosen so low that substantial ion pair extraction effects (17,18) are suppressed. Thus, for the shake bottle method,  $\log P = -3$  seems to be the lower measuring limit in all cases. Thus, for substances, which possess more than one charged group and for which very low log P's are expected from calculation, experimental measurements appear superfluous because they provide no additional information.

3. Ion pair extraction (17,18) may, however, play a role which cannot be neglected, if the concentration of a chemical and/or its counter ion becomes large in the water phase. Our experiments (11) have shown that a substantial increase of the octanol/water partition coefficient

FIG. 1 Relation between measured and calculated partition coefficients of 19 dyes



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of anionic or cationic dyes is observed when sodium chloride is added to the aqueous phase. If sodium chloride is added at physiological concentration and the dye concentration is in the range of 0.1 to 1  $\mu\text{mol/l}$  an increase of  $\log P$  by up to 2 units may be observed.

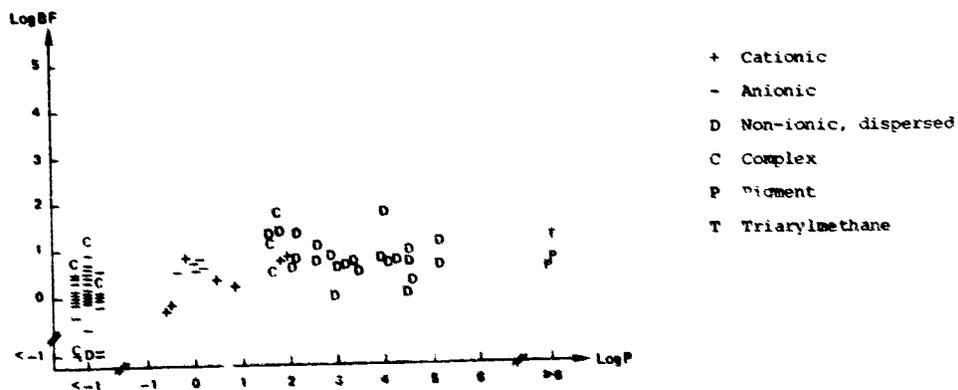
These observations suggest that a higher bioaccumulation of ionic dyes may occur in sea water fish than in fresh water fish under similar conditions. This possibility warrants consideration however only if  $\log P > 1$  (for pure water), which would be unusual for an ionic dyestuff.

The calculated partition coefficient seems in most cases to be a fair predictor for the measured  $\log P$  provided the calculation is carried out with expertise and with proper application of all rules associated with the fragment methods. This procedure allows prediction of  $\log P$  with an uncertainty of less than two powers of ten and is undoubtedly more valuable than an experiment in the cases of very hydrophilic ( $\log P < -2$ ) or very lipophilic ( $\log P > 5$ ) compounds where the possibilities of experimental errors are obviously very high.

#### Estimation of bioaccumulation factors from $\log P$

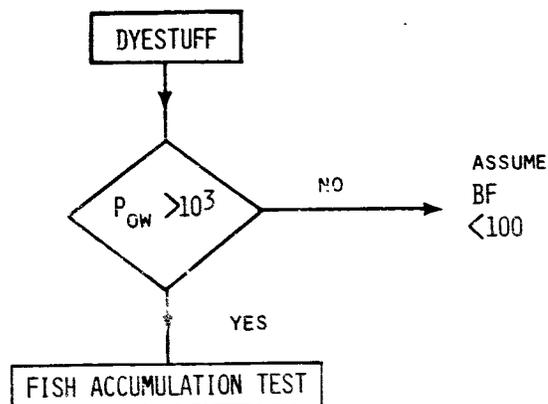
Fig. 2 shows the relationship between the bioaccumulation factor and  $\log P$  (where available, experimental  $\log P$  values were used). It is not the intent of this paper to confirm the correlation between  $\log BF$  and  $\log P$  as established in several recent papers (2,3,19-21), but rather to distinguish between those products for which a fish accumulation test is justified and those for which it is superfluous. Although these data relate to products which have been tested and shown not to bioaccumulate to an unacceptable extent, no dyestuffs have been found so far which have a  $BF > 100$  and an  $P < 1000$ , and which would, therefore, be exceptions to the proposed OECD scheme (Fig. 3) (11).

FIG. 2 Relation between  $BF$  in fish and  $P_{O/W}$  of 72 dyestuffs



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FIG. 3 Simplified version of test flow scheme presented in ref. 11 and discussed within the OECD Chemicals Testing Programme



Some interesting features of these data may be usefully discussed in terms of the various classes of colorants:

Ionic dyestuffs (e.g. Acid, Basic, Direct and Reactive Dyes)

With few exceptions even the very hydrophilic dyestuffs showed a BF of 0.1-10 although from P lower BF's would have been predicted (3,19-21). It is probable that this is due to dyestuff adhering to the outside of the fish or to the intestines: the accumulation test involves measurement of dyestuff in the whole fish whereas the partition coefficient is more strictly a predictor of accumulation tendency in fish fat.

None of the dyestuffs bearing at least one charged group showed a BF  $> 10$  and for such products a fish accumulation test is superfluous. For such a product to have a calculated  $\log P > 3$ , assuming a charge contribution of  $-5$  log units, could only arise if the rest of the structure is extremely lipophilic ( $\log P > 8$ ). Such a product would be quite exceptional and would exhibit other extraordinary properties, e.g. strong surface activity or insolubility, and on the basis of its  $\log P > 3$  would anyhow be subjected to a fish accumulation test.

The applicability of P as a predictor of bioaccumulation has been questioned in the case of ionic compounds (23). The presented results indicate that this reservation is not justified in the case of ionic dyestuffs.

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#### Metal complex dyestuffs

The partitioning behaviour of metal complex dyestuffs is not well understood and there is little information in the literature. Our experiments so far have shown that the measured log P of the whole complex corresponds to that which would be predicted from the lipophilicity of the organic ligand alone. The ligands of three measured complexes contain sulphonamide or carboxyl groups, which become negatively charged upon complexation. Thus, the metal complex as a whole carries no net charge in spite of the presence of the positively charged metal cations. Further work on carefully purified metal complex dyestuffs will be necessary to predict their partitioning behaviour more accurately.

#### Disperse dyestuffs

Disperse dyestuffs have only a low solubility in water. Fig. 2 includes several Disperse dyestuffs which have been demonstrated not to bioaccumulate in fish even though their log P values are greater than 3. This behaviour is quite different from that of the halogenated hydrocarbon insecticides such as DDT, which show a high BF in spite of very low solubilities: for DDT BF is 84,500 in the static fish test and the water solubility is 1,7 ppb (19). The precise reason for this difference is not known, but may be due to the relatively high molecular weight (ca. 450-550) of Disperse dyestuffs making transport across the fish membranes difficult.

In any case under the proposed OECD scheme, these Disperse dyestuffs would, as their log P exceeds 3, be subjected to a fish accumulation test.

#### Organic pigments

Of particular interest is the observation, that even the three most lipophilic compounds tested, the triarylmethane dye base no. 12 in Table 1, Part A, and the two pigments no. 45 and 46 in Table 1, Part B, all show bioaccumulation factors well below 100, i.e. between 3 and 15. On the basis of their calculated partition coefficients of log P = 11.0, 9.5 and 9.3, respectively, one would, of course, predict accumulation factors substantially above  $10^6$  or even  $10^7$ , using any of the established correlation equations between log BF and log P (2,3,19-21). The absence of bioaccumulation of these pigments is presumably due to their extremely low water solubility, possibly coupled with a molecular weight effect as suggested for Disperse dyestuffs. These low solubility effects are further enhanced by the fact, that dissolution rates for extremely insoluble hydrophobic solids are usually very low (22), so that equilibration with water may take months or even years. For compound no.12, for instance, water solubility has been estimated to be less than  $10^{-12}$  g/l, and there will certainly be no measurable uptake of truly dissolved compounds into the living organism possible within the duration of the fish test. The same holds true for the two pigments, which also have extremely low water solubilities.

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### CONCLUSIONS

1. Practical experience indicates that for a wide range of ionic dyestuffs, the partition coefficient in n-octanol/water, P, provides a useful indicator of bioaccumulation tendency.
2. Regardless of the ionic or non-ionic nature of the substance, provided  $P_{exp} < 1000$ , it may be confidently predicted that the BF in the fish test will be  $< 100$ , and that the carrying out of this expensive test is superfluous.
3. For extremely lipophilic or hydrophilic products, the P cannot be determined experimentally. The calculation procedure for P provides a satisfactory alternative approach. If  $P_{calc} < 0.1$ , the experimental determination of P, as well as the fish accumulation test, is unnecessary.
4. The calculation of P is useful as a check of the experimental value and provides also a basis for selecting the appropriate method for the experimental determination.

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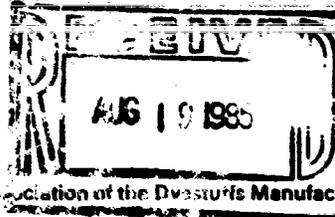
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Washington, D.C. 20460

Your Ref.

Our Ref. RA

CH-4005 Basel, August 14, 1985

AUG 22 RECD  
DMS

Dear Sirs,

OPTS-41017

Comments and Information on C.I. Pigment Green 7 (CAS No. 1328-53-6)  
Placement in the Intent-To-Designate Category

In its note in 50 Fed. Reg. 13418-20 the Interagency Testing Committee (ITC) has announced that it intends to designate Pigment Green 7 for chemical fate and ecological effects testing under Section 4(a) of TSCA and requests the submission of specific information with respect to that chemical.

The Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD) is a worldwide industry association with Member Companies in Europe, Japan, India, and the U.S.A. In the United States ETAD is represented by the U.S. Operating Committee of ETAD (USOC). Whereas on the international basis, ETAD concerns itself with both dyestuffs and organic pigments, within the U.S. it represents the dyestuffs industry only of the U.S. members, as pigment manufacturers are already represented in the U.S. by the Dry Color Manufacturers' Association (DCMA).

ITC is asking for available information on the water solubility, acute toxicity to fish, aquatic invertebrates and algae of Pigment Green 7.

ETAD has just completed a paper which will include some data on Pigment Green 7 requested by ITC. Although not yet published, ETAD will provide ITC with these data including the full manuscript of the paper. The data can become part of the public record of the ITC review process whereas the full paper should not be distributed before its appearance in the scientific journal "Chemosphere". The following comments are intended to set forth reasons which would suggest to exclude Pigment Green 7 from further testing.

# ETAD

In its extensive and continuing studies on the fate of organic colorants (i.e. dyestuffs and organic pigments) ETAD concluded also investigations to generate data as a basis for assessing the bioaccumulation potential of colorants in fish. It could be experimentally shown for a wide range of ionic and non-ionic dyestuffs that the partition coefficient in n-octanol/water  $P_{ow}$  provides a useful indicator of bioaccumulation tendency. If the  $P_{ow}$  is  $< 1000$ , it can confidently be predicted that the bioaccumulation factor (BF) in fish will be  $< 100$  i.e. the chemical is not considered to be bioaccumulating. So far results of fish accumulation tests with over 75 dyestuffs have confirmed this empirical rule (Appendix A).

For organic pigments with their extreme solubility characteristics, i.e. extremely low water solubility combined with a very low solubility in organic solvents another approach had to be taken mainly due to the following factors:

1. The experimental determination of  $P_{ow}$  of such insoluble pigments can be extremely difficult or even practically not possible.
2. The very high calculated  $P_{ow}$ -values would theoretically predict a strong bioaccumulation tendency. Despite of this all the fish accumulation tests performed with pigments did not reveal any tendency for any substantial storage of these substances in fish.

Studies to investigate this phenomenon have recently been completed, and the manuscript of a paper reporting the results will be submitted for publication in Chemosphere shortly (Appendix B). It is concluded that in the case of pigments with very low solubilities the log BF vs log  $P_{ow}$  correlation equations are not suited for the assessment of their bioaccumulation. A better approach is to determine the n-octanol solubility which is fairly well comparable with the solubilities in fat (lipids). Based on this value which allows the calculation of the maximum possible storage in fish, and other parameters including calculated water solubility, amounts introduced into the aquatic environment, ecotoxicological and toxicological properties, a risk assessment can be made.

The study includes also Pigment Green 7 (compound No. XX in Appendix B) and the following data have been determined:

Experimental solubility $C^0$ in n-octanol at 20°C:	0.07 mg/l
Calculated solubility $C_s^{ws}$ in water at 20°C	: $7 \cdot 10^{-18}$ to $2 \cdot 10^{-16}$ mg/l
log $P_{ow}$ calculated	: 17.40

Based on the solubility values it can confidently be concluded that this pigment is not bioavailable. This is supported by the experience with the compounds No. XIV, XV and XVI, the Diarylid Yellow Pigments, with similar solubility characteristics and which did not show any toxic effects in highly dosed 2-year bioassays nor any sign of metabolic conversion (see e.g. cited literature in references 17, 18 and 20 of Appendix B).

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Conclusion

Considering this lack of bioavailability, the inability to build up any substantial amounts in fish, the very small amounts of Pigment Green 7 entering the aquatic environment and the demonstrated very low acute and chronic toxicity, the environmental risk from Pigment Green 7 is so small and trivial that further testing does not seem to be warranted.

Please do not hesitate to contact us if you think we can be of any further assistance.

Respectfully submitted,

Encls.

ETAD

*R. Anliker*  
Dr. R. Anliker  
Executive Secretary

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