Some Physical Factors in Toxicological Assessment Tests

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Many thousands of organic compounds are in common use and new ones introduced daily. With many of these materials, little is known about their toxic hazard. For years scientists have been investigating the relation of structure and properties to biological activity. Among the factors relating to toxicity are bioaccumulation and persistence in the organism. In this study, the relation of partition coefficient and solubility to bioaccumulation of some organochlorine compounds was investigated as was also the reactivity of several organophosphates. The work adds confirmation to the relation of molecular parameters to penetration, accumulation, and persistence in toxic action.

Introduction

Some 30,000 or more chemicals of varying types are in common use. They range over the spectrum of simple inorganic compounds through metallic-organic to very complex organic molecules and polymers (1).

Just as the chemicals vary widely, so do the uses. One substance may be used for a solvent, another as a pesticide, and a third as a plastic. Some chemicals are used individually, others in complex formulations. The use of many of these chemicals result in their introduction to the environment, with the consequent exposure of man to the substance.

It is an axiom of toxicology that any substance in sufficient concentration can be injurious. Just so, there is reason to believe that many of the chemicals in use may afford a greater or lesser hazard to man upon prolonged exposure. Whether or not the hazard is significant can be determined only on the basis of some knowledge of the toxicity of the compound and the levels to which exposure occurs and the interaction of environmental factors (2). This requires information about the bioavailability, rate of uptake, accumulation, persistence, metabolism, and excretion, as well as the basic mechanism by which the effect is produced. Such information is available for only a relatively few of the some 30,000 compounds in use.

If the toxicity and hazard of these chemicals were to be evaluated in time to protect man, there is needed (a) a rapid method of measuring toxicity; and (b) a method of predicting probable toxic hazard of new or as yet untested compounds (3, 4). In view of the magnitude of the problem, both are needed, the first to give empirical data that can be extrapolated to man and allow the setting of standards or quality criteria that would avoid toxic consequences. The second method, namely that of predicting probable hazard, would provide early warning with new or as yet untested compounds, point to methods of handling such compounds, and further, provide guidance in the development of rapid assessment of toxicity by empirical means. It is to this latter approach to evaluating toxicity that this paper is addressed.

Background

Since the 19th century, investigators have been intrigued by the problem of relating biological activity to structure and properties of chemicals (5, 6). The interest was stimulated by the new and novel compounds, the young science of organic chemistry produced for pharmaceutical testing. Shortly, of course, wider interest in biological activity and toxicity developed as these compounds were put to other purposes, e.g., pesticides, or as a result of effect on man from industrial exposure. It was felt that relating biological activity to molecular parameters offered the advantage of being able to predict biological activity and toxicity on the basis of rela-
tively rapidly obtainable chemical data, and that it would enable the "tailor-making" of desirable compounds or early recognition of possibly hazardous substances.

Many scientists over the years have devoted attention to various aspects of the problem of predicting biological activity from structure and properties of compounds. Some of the earliest studies involved examination of the structure of organic compounds in relation to their activity. In these early studies, particular attention was given to the composition and substituent groups of compounds including such things as halogen, nitro, alkyl, amino, thio, and mercapto substitution. This enabled investigators to identify groupings within molecules that conferred a measure of biological activity, e.g., chlorine substitution on aromatic compounds. Paralleling these studies were investigations attempting to relate various other properties to biological activity, including such things as boiling point, vapor pressure, solubilities, partition coefficients, molar volumes, and polarizability. Limited successes were achieved in these early studies as evidenced by the information that was developed enabling prediction of greater or lesser biological activity based on organic structure, positional isomerization, and the nature of the substituent group. For the most part, however, this information was applicable only within specific homologous series and rarely could be extended to another class of organic.

More and more successes have been recorded in relating structure and properties to biological activity with application of newer and more sophisticated knowledge and techniques in studying the problem. Moreover, the wealth of information developed over the years relating a specific type of activity with a given group or region of composition in a molecule has been of value. The application of quantum mechanical concepts in relating structure to activity has been fruitful on certain types of problems (6–9). Thus, application of the results of molecular orbital calculations has revealed certain characteristics of the molecules that correlate well with activity. Similarly, determination of electron density in regions of molecules has been shown to correlate well with oncogenic activity with certain types of chemicals (10). Other factors that have been studied and shown to correlate to a greater or lesser degree with biological activity include symmetry of the molecule, electromagnetic absorption, particularly infrared, the activity and the constitutive property of partitioning.

It becomes increasingly apparent that the biological activity (or toxicity) of a chemical is the sum of a variety of molecular characteristics interacting to a varying degree in the several events leading up to the basic reaction. These characteristics can be shown to include the composition and configuration of the molecule, isomerism, spatial geometry, and the various thermodynamic properties that determine the constitutive and colligative characteristics. Analyses of the processes and events leading to the expression of toxicity provides some insight as to the various factors, molecular and otherwise, and the role they play in biological activity. The "critical path" of a chemical to an interaction with the target site is seen as involving four components or events, namely, (1) movement through and interaction of the chemical with the environment; (2) interaction of the chemical with the boundary between the organism and the environment; (3) passage of the drug through the boundary, i.e., absorption and diffusion; and (4) intracellular action of the drug. In each of these steps, several reactions may occur. Certain of these reactions may favor increased intracellular concentration, others tending to limit it.

Having reached the boundary of the organism, interaction with the boundary and passage through it ensues. This is followed by transport and distribution within the tissues themselves until a biologically significant concentration is reached at the sensitive site.

Obviously, the flux of the chemical to the target site is influenced by both the character of the system as well as the molecular properties of the chemical. This is true whether the processes are enzymatically mediated or purely physical. Since the interest this instance is on the chemical, the analyses, then, should focus on its characteristics. Table I is an attempt at just such analyses.

In the course of the work of this cooperative study between the US and USSR health scientists, it became of interest to investigate some of the physicochemical properties in relation to their role in biological activity. The purpose was to further the understanding of molecular properties, both chemical and physical, as a basis for making predictions regarding toxicity. Various factors were examined as they related to the potential for accumulation, persistence, and ultimately the toxic action. Findings, it was felt, apply not only to predicting toxicity of new or untested compounds but are also of value in developing appropriate, rapid toxicity assay methods and in setting standards.

Materials and Methods

Materials

The chemicals utilized for the most part were analytical standard grade of greater than 95% pur-
Table 1. Processes and molecular properties in relation to biological activity.

| Site                          | Process                  | Molecular properties implicated                                      |
|-------------------------------|--------------------------|---------------------------------------------------------------------|
| Environment                   | Transport                | Solubility                                                          |
|                               |                          | Vapor pressure                                                      |
|                               |                          | $\Delta H$ (solution)                                               |
| Interaction                   |                          | Partition coefficient                                               |
|                               |                          | Molar polarization                                                  |
|                               |                          | Dimensions and Spatial Geometry                                      |
|                               |                          | Reactivity                                                          |
|                               |                          | Photochemical                                                       |
|                               |                          | Hydrolysis                                                          |
|                               |                          | Oxidation/reduction                                                 |
|                               |                          | Complexation                                                        |
| Boundary of organism          | Interaction              | Dipole, van der Waals, and chemical binding                        |
|                               |                          | Reactivity                                                          |
|                               |                          | Partial molar volume                                                |
|                               |                          | Fugacity                                                            |
|                               |                          | Symmetry/size                                                       |
|                               |                          | Partition coefficient                                               |
| Inter- and intracellular      | Transport                | Solubility/partitioning                                             |
|                               |                          | Polarizability                                                      |
|                               |                          | Dipole                                                              |
|                               |                          | Diffusivity                                                         |
|                               |                          | Molal volumes                                                       |
|                               | Interaction              | Dimension/geometry                                                  |
|                               |                          | Bond energies/reactivity                                            |
|                               |                          | Composition                                                         |
| "Active Site"                 | Interaction              | Hydrogen bonding, polarizability, partition coefficient              |
|                               |                          | Reactivity/bond energies                                            |
|                               |                          | "Activation"                                                       |
|                               |                          | covalent bonds                                                      |
|                               |                          | Size, geometry, orientation, dimension between bonding sites, exclusion volume |

ity. The other chemicals, such as solvents and salts, were reagent grade or purified before use. All water utilized was distilled and run through a $2 \times 10$ cm XAD-2 macroreticular resin column. This effectively removed trace organics from the water. The preliminary studies indicated the reagent grade octanol was unsuitable for partitioning work, possibly due to trace impurities which serve to stabilize the emulsion formed at the octanol/water interface. It was found that a number of these impurities could be removed by distillations.

Analyses

All samples were analyzed by gas/liquid chromatography, using an $^{60}$Ni electron capture detector operating in the pulse mode. Glass columns act with the appropriately coated solid phase for each type of chemical used. Oven temperatures and gas flow rates found suitable in the prior work for the compounds were used as the operating conditions.

Hydrolysis

The rates of hydrolysis were determined using a modification of the method described by Ruzicka et al. (11). An amount of chemical approximately equal to half the aqueous solubility limit in 100 ml deposited on the walls of the flask by evaporating off an ether solution. After the residual ether was removed by a nitrogen stream, flasks were filled with an aqueous buffer solution. The phosphate buffer was $0.008695M \text{KH}_2\text{PO}_4$ and $0.03043M \text{Na}_2\text{HPO}_4$ having a pH of 7.4. The flasks were shaken vigorously for 5 min and several aliquots immediately removed for zero time analyses. Samples of the chemical in the buffer solution were maintained at $37.5 \pm 1^\circ C$ and at $20 \pm 1^\circ C$ and the hydrolysis rate followed. The concentration at any serial time was determined by analyses for the remaining amount of parent compound. The half-life was then determined from a first-order rate plot and the enthalpy of activation for hydrolysis calculated.
from the differences in rate between the two temperatures.

**Partition Coefficient**

Stock solutions of appropriate concentration usually about 1 mg/ml in octanol were prepared. A 2-ml portion of the stock solution was added to 20 ml organic-free distilled water in a screw-top (Teflon-lined) 25 ml Corex centrifuge tube. Tubes were shaken in horizontal position for 24 hr at 20°C, and then 1 ml of the octanol solution was removed for analyses. The remaining octanol was withdrawn along with the top few milliliters of the aqueous phase and discarded. The remaining aqueous phase was centrifuged for 20 min (17,500 rpm, approximately 39,000g) in a Servall refrigerated centrifuge at 20°C. An additional few milliliters was again discarded to remove octanol separated by centrifugation and a 10 ml sample withdrawn for analyses. The samples were then diluted (octanol) or extracted with hexane to the correct volume for analyses by gas-liquid chromatography (GLC).

**Solubility**

Sufficient chemical to be approximately five times the estimated water solubility was evaporated onto the walls of a 1 liter Erlenmeyer flask from an ether solution (1/2). The flasks were filled with organic-free distilled water and fitted with an inverted fritted gas dispersion tube. The tube was attached to a Teflon stopcock to facilitate removal of aliquots of the solution. The second tube, not extending below the liquid level, allowed the use of air pressure to remove the sample through the dispersion tube for analyses. The flasks were magnetically stirred and samples removed for analyses at regular intervals. The sampling continued until five consecutive samples with less than 5% variation in concentration were obtained. The solubility given is the average of these five samples. A visible excess of all compounds remained when the determinations were terminated.

**Results and Discussion**

The solubilities of organochlorine and organophosphate compounds used in this study are shown in Tables 2 and 3. Included in these tables also is the logarithm of the partition coefficient.

### Table 2. Solubilities and partition coefficients (octanol/water) of organophosphates.

| Compound       | Solubility in water, ppm | log $P$ |
|----------------|--------------------------|---------|
| Phosmet        | 25                       | 2.83    |
| Fenitrothion   | 30                       | 3.38    |
| Dicophosphon   | 6.25                     | 3.58    |
| Parathion      | 11.9                     | 3.81    |
| Phosalone      | 2.15                     | 4.30    |
| Methyl chlorpyrifos | 4.76              | 4.31    |
| Dialilor       | 0.18                     | 4.69    |
| Ronnel         | 1.08                     | 4.88    |
| Chlorpyrifos   | 0.4                      | 5.11    |
| Dichlofenthion | 0.245                    | 5.14    |
| Leptophos      | 0.0047                   | 6.31    |

### Table 3. Solubilities and partition coefficients of various compounds.

| Compound                  | Solubility in water, ppm | Log ($n$-octanol/H$_2$O partition coefficient) |
|---------------------------|--------------------------|-----------------------------------------------|
| Benzene                   | 1710 (20°C)              | 2.13                                          |
| Toluene                   | 470 (16°C)               | 2.69                                          |
| Fluorobenzene             | 1540 (30°C)              | 5.27                                          |
| Chlorobenzene             | 448 (30°C)               | 2.84                                          |
| Bromobenzene              | 446 (30°C)               | 2.99                                          |
| Iodobenzene               | 340 (30°C)               | 3.25                                          |
| $p$-Dichlorobenzene       | 79 (25°C)                | 3.38                                          |
| Naphtalene                | 30                       | 3.37                                          |
| Diphenyl ether            | 21 (25°C)                | 4.20                                          |
| Tetrachloroethylene       | 400 (25°C)               | 2.60                                          |
| Chloroform                | 7950 (25°C)              | 1.97                                          |
| Carbon tetrachloride      | 800                      | 2.64                                          |
| p,p'-DDT                  | 0.0031-0.0034 (25°C)     | 6.19                                          |
| p,p'-DDE                  | 0.04 (20°C)              | 5.69                                          |
| Benzoic acid              | 2700 (18°C)              | 1.87                                          |
| Salicylic acid            | 1800 (20°C)              | 2.26                                          |
| Phenylacetic acid         | 16,000 (20°C)            | 1.41                                          |
| Phenoxyacetic acid        | 12,000 (10°C)            | 1.26                                          |
| 2,4-D                     | 890 (25°C)               | 2.81                                          |
| 2,4,5,2',5'-PCB           | 0.010 (24°C)             | 6.11                                          |
| 2,4,5,2',4',5'-PCB        | 0.00095 (24°C)           | 6.72                                          |
| 4,4'-PCB                  | 0.062 (20°C)             | 5.58                                          |
The solubilities presented in these tables are the best values either obtained experimentally or from the literature. The solubility behavior for the most part is about what would be expected with more complex and less polar compounds showing a reduced water solubility. In the case of the organophosphates, the larger the alkyl group (ethyl versus methyl) the lower the solubility. Conversely, the partition coefficient is larger. It would be expected, therefore, that these compounds might more readily partition through lipophilic membranes and thus gain more ready access to the interior of the cell and to the target site (13, 14).

Figure 1 is a plot of the log of the water solubility versus the logarithm of the partition coefficient. Despite some differences in temperatures at which solubilities were determined, there is an excellent correlation between the partition coefficient and water solubilities for the wide range of compounds studied. An inverse linear relationship in the log-log plot is noted, and this relationship obeys the regression equation:

$$\log K = -0.670 \log S + 5.00$$

The correlation coefficient of this relationship is 0.985.

As a further assessment of the validity of the relation between partition coefficient and accumulation, these two factors were compared (13, 14). Table 4 presents limited number of data of partition coefficient and bioaccumulation. Though the data are limited, the correlation is obvious.

A plot of these data shows a linear relationship with a correlation coefficient of 0.983. The regression equation relating the dependence of bioconcentration on aqueous solubility is:

$$\log B = -0.624 \log S + 3.72$$

Though the persistence of a compound in the mammalian body is influenced by deposition, excretion, and enzyme-mediated metabolism, it was felt that the rate of hydrolysis in physiological pH buffers would give an indication of possible persistence. This persistence would relate to the organism's ability to accumulate and store the material, and hence acquire sufficiently high concentration for prolonged exposure. Table 5 gives the results of studies of the hydrolysis of several of the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Relationship between the partition coefficients and aqueous solubilities of various organic compounds.
organophosphates. The organochlorines were not studied in this connection because of the known stability and resistance to hydrolysis. Further proof of the relation of partition coefficient indicate accumulation and potential toxicity was found with dichlofenthion and leptoephos. Here the individual had a single oral exposure but symptoms of intoxication and high residue levels upon biopsy were found for 30 + days following the exposure (15). Leptoephos, of course, has a relatively low acute toxicity in short-term tests but has been demonstrated to be neurotoxic as a result of its accumulation and persistence.

Summary and Conclusions

It is shown that a variety of molecular parameters are involved in the penetration, accumulation, persistence, and toxic action of a chemical. The solubility-partitioning is an important factor in penetration and accumulation. It also appears to have a significant relationship in terms of indicating the intrinsic toxicity. Applying this information further, these properties can be shown to be related to biological activity through the relationship \( BA = \log S/S_0 \).

It is concluded on the basis of experiment and observation that persistence is a significant factor in toxic action. If the basic molecular moiety has particular stability—except for activation reactions—it is highly probable that such a compound will exhibit chronic effects. This is illustrated in the case both in the case of the organochlorines and the more stable organophosphates.

From these data and observations it is concluded that toxicity is the algebraic sum of the interaction of a number of molecular parameters.

REFERENCES

1. N. S. F. Chemicals and Health. Report of Panel on Chemicals and Health, the President’s Science Advisory Committee, National Science Foundation, September 1973.

2. Fouts, J. R. Overview of the field: environmental factors affecting chemical or drug effects in animals. Fed. Proc. 35: 1162 (1976).

3. Shigan, S. S. Methods for predicting chronic toxicity parameters of substances in the area of water hygiene. Environ. Health Perspect. 13: 83 (1976).

4. Stoltz, D. R., Poirier, L. A., Irving, C. C., Stich, H. F., Weisburger, J. H., and Grice, H. C. Evaluation of short-term tests for carcinogenicity. Toxicol. Appl. Pharmacol. 29: 157 (1974).

5. Albert, A. Selective Toxicity, 4th Ed., Methuen, London, 1968.

6. Fujita, T. The extrathermodynamic structure–activity correlations. In: Biological Correlations—The Hansch Approach. Adv. Chem. Ser. 114, American Chemical Society, Washington, D. C., 1972.

7. Cohen, J. L., Lee, W., and Lien, E. J. Dependence of toxicity on molecular structure: group theory analyses. J. Pharm. Sci. 63: 1068 (1974).

8. Hansch, C., Quinlan, J. E., and Lawrence, G. L. The linear free energy relationship between partition coefficients and aqueous solubility of organic liquids. J. Org. Chem. 33: 347 (1968).

9. Verloop, A. The use of linear free energy parameters and other experimental constants in structure activity studies. In: Drug Design, E. J. Ariens, Ed., Academic Press, New York, Vol. 3, 1972, p. 133.

10. Thurndon, W. C. The theory of carcinogenic activity of aromatic hydrocarbons. Trans. N. Y. Acad. Sci. 36: 200 (1974).

11. Ruzicka, J. H., Thomson, J., and Wheals, B. B. The gas chromatographic determination of organophosphorus pesticides. II. A comparative study of hydrolysis rates. J. Chromatogr. 31: 37 (1967).

12. Haque, R., and Schmedding, D. A method of measuring the water solubility of hydrophobic chemicals: solubility of five polychlorinated biphenyls. Bull. Environ. Contam. Toxicol. 14: 13 (1975).

13. Kenaga, E. E. Partitioning and uptake in biological systems. In: Environmental Dynamics of Pesticides, R. Haque and V. H. Freed, Eds., Plenum Press, New York, 1975.

14. Lu, P. Y., and Metcalf, R. L. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. Environ. Health Perspect. 10: 269 (1975).

15. Davies, J. E., Barquet, A., Freed, V. H., Haque, R., Morgade, C., Sonneborn, R. E., and Vlackev, C. Human pesticide poisoning by a fat-soluble organophosphate insecticide. Arch. Environ. Health 30: 608 (1975).