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13. ABSTRACT *(Maximum 200 words)*

A quantitative structure-activity relationship study was performed on 33 chlorinated alicyclic compounds, most of which are chlorinated cyclodiene insecticides or their structural analogs. Their action on the t-butylbicyclophosphorothionate (TBPS) binding site of the gammaaminobutyric acid (GABA) receptor/chloride ionophore complex is being correlated with their lipophilicity, electronegativity and/or molecular connectivity characteristics. The biological parameters were investigated at Mississippi State University. The in vitro potency of the test compounds to compete with [^{33}S] TBPS for binding to rat brain membranes was studied. Also the potency of the test compounds to inhibit 36Cl-flux into rat brain vesicles is being studied. The three chemical parameters were investigated at Iowa State Unviersity. Nuclear magnetic resonance was used to evaluate the electronic character of the alicyclic hydrocarbons being studied. Partition coefficients were determined for the series of compounds. Topological characteristics were elucidated using molecular connectivity indices.

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Report 91-0338

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS OF CHLORINATED ALICYCLIC COMPOUNDS

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March 14, 1995

Final technical report for period July 1, 1991 - October 30, 1994.

FINAL TECHNICAL REPORT

STATEMENT OF WORK

A quantitative structure-activity relationship study was performed on 33 chlorinated alicyclic compounds, most of which are chlorinated cyclodiene insecticides or their structural analogs. Their action on the *t*-butylbicyclophosphorothionate (TBPS) binding site of the gamma-aminobutyric acid (GABA) receptor/chloride ionophore complex is being correlated with their lipophilicity, electronegativity and/or molecular connectivity characteristics.

The biological parameters were investigated at Mississippi State University. The *in vitro* potency of the test compounds to compete with [^{35}S]TBPS for binding to rat brain membranes was studied. Also the potency of the test compounds to inhibit ^{36}Cl- flux into rat brain vesicles is being studied.

The three chemical parameters were investigated at Iowa State University. Nuclear magnetic resonance was used to evaluate the electronic character of the alicyclic hydrocarbons being studied. Partition coefficients were determined for the series of compounds. Topological characteristics were elucidated using molecular connectivity indices.

STATUS OF THE RESEARCH

The research project was designed to conduct a quantitative structure-activity relationship study on an extensive set of chlorinated alicyclic compounds. The majority of the compounds were chlorinated cyclodiene insecticides or model compounds similar to them. The chlorinated cyclodiene insecticides were widely used in the United States for a number of years, but are no longer approved for use by the Environmental Protection Agency because of their stability, lipophilicity and persistence. Historical residues of these organochlorine insecticides would be expected to be present in the US despite the fact that they have been banned for 15-20 years. They are still in use in some other areas of the world; therefore, it is reasonable to expect that human exposure to some of these compounds will be occurring even today. Some of these insecticides display a high acute mammalian toxicity, adding to the potential hazard.

The large series of compounds which were available for study lent themselves to a structure-activity relationship which promised to be detailed and meaningful. The current assumption on the mechanism of action on the chlorinated cyclodiene insecticides is an interference with the integrity of GABA receptor function. The compounds are documented to compete with TBPS for binding at the GABA receptor. The result of binding is to restrict chloride flux, making the neuron less stable, leading to depolarization and hyperactivity within

the nervous system. At sufficiently high doses, the results of exposure can be fatal. There is a substantial range in the acute toxicity levels displayed by the chlorinated cyclodiene insecticides.

The binding of [^{35}S]TBPS to rat brain membranes revealed a typical saturation curve for binding which suggested a single population of receptors. A Kd of 24.6 nM was calculated, which is consistent with literature reports. All compounds except mirex inhibited the binding of [^{35}S]TBPS, and displayed a very wide range of potencies. IC_{50}'s for the compounds were 4.32-18,475 nM. Since most of the compounds are model compounds, there is no acute toxicity data available for them. However, the acute toxicity levels of those known do correlate well ($r=0.940$) with the inhibitory potencies. This correlation suggests that the presumed mechanism of action on the TBPS site of the GABA receptor is the cause of toxicity, and that dispositional and metabolic factors do not substantially influence the toxicity. The chloride flux assays have proven more problematical than anticipated, and are still in progress. However, the QSAR has been conducted on the inhibition of TBPS binding, which appears to be suitable index of the potency of action of these organochlorine compounds.

Lipophilicity was assessed by a high performance liquid chromatography method. These organochlorine compounds are highly lipophilic compounds. The lipophilicity does not correlate with neurochemical potency overall. However, if the compounds are subdivided into groups, mainly on the presence or absence of an oxygen, the correlation is far better. Likewise, molecular connectivity also correlated with potency if the compounds were grouped into similar structures. Clear relationships exist between the zero, first and second order indices and the activity of the compounds in the TBPS-binding assay. Chemical shift data in the proton NMR did not show significant relationships with the biochemical data.

In summary, the potency of the test compounds to displace the binding of [^{35}S]TBPS to rat brain membranes correlates well with the acute toxicity levels of compounds for which LD_{50}'s have been reported. These correlations indicate that, for the majority of the test compounds, i.e., those displaying the chlorinated cyclodiene-type structure, the interference with GABA receptor function is probably their primary acute neurotoxic action. The diversity of IC_{50}'s suggests that the compounds yield a wide range of biological potencies. The biological potency can be largely predicted within chemically similar groups by lipophilicity and molecular connectivity, but not by electronic character.

PUBLICATIONS

Ma, T., J. Tang and J.E. Chambers. 1992. The relationship of the neurochemical actions of chlorinated alicyclic compounds to acute toxicity. Society of Environmental Toxicology and Chemistry Abstracts, WA6G8, p. 228.

The above paper was also presented to the South Central Chapter of the Society of Toxicology meeting, and the abstract was published in the meeting program.

Liu, J., J. E. Chambers and J. R. Coats. 1994. Determination of lipophilicity of chlorinated alicyclic compounds by reversed-phase high performance liquid chromatography. J. Liquid Chromatography 17:1995-2004.

Abstracts:

Chambers, J.E., J. Tang, T. Ma, J. Liu, and J.R. Coats. 1993. Structure-activity relationships of chlorinated alicyclic compounds on gamma aminobutyric acid receptors in rat brain. SETAC Abstract P311, P. 218. Presented: Society of Environmental Toxicology and Chemistry, Houston, Texas.

Carr, R.L., T.T. Atterberry, and J.E. Chambers. 1994. The interaction of chlorinated alicyclic insecticides with brain GABA receptors in channel catfish (*Ictalurus punctatus*). Toxicologist 14:917. Presented: Society of Toxicology, Dallas, Texas.

PERSONNEL

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Jun Tang, B.S., Graduate Student
Russell L. Carr, Ph.D., Graduate Student and Post-doctoral Associate

Iowa State University
Joel R. Coats, Ph.D., Co-Investigator
Jainbo Liu, M.S.. Graduate Student

INTERACTIONS

None.

NEW DISCOVERIES

None.

OTHER INFORMATION

This report covers the entire project period. Since the chloride flux data is incomplete, the QSAR using this parameter is incomplete. We are working on these at present and will supply AFOSR with the results as soon as the analysis is complete. We are also currently working on additional publications, and we will supply these also as they are completed.

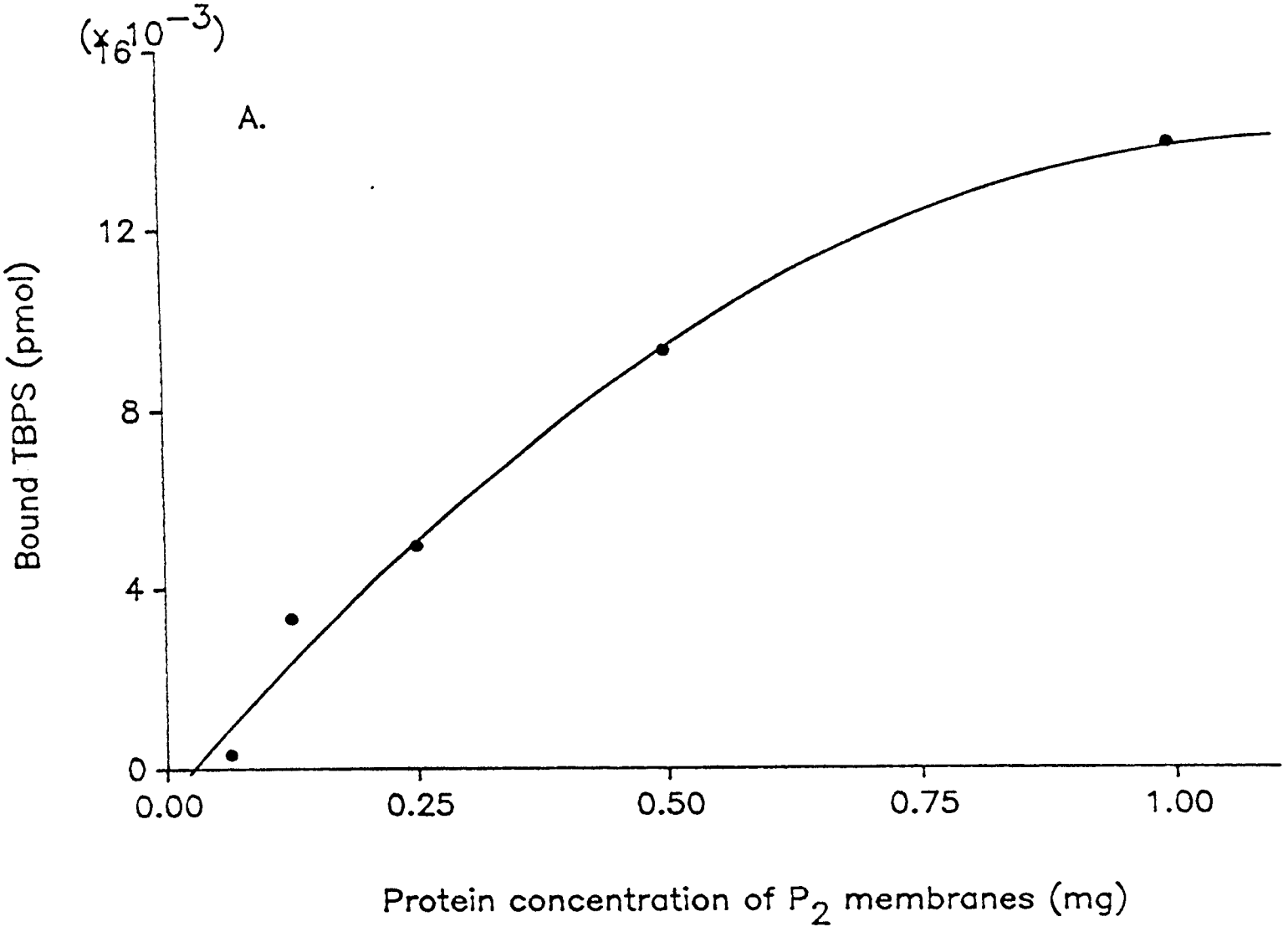
TABLE 2

INHIBITION OF TBPS BINDING TO RAT BRAIN GABA RECEPTORS BY VARIOUS ORGANOCHLORINE COMPOUNDS

Compound	IC_{50}[1] (nM)			
12-keto Endrin	4.32	±	0.27	(3)
Photoheptachlor	5.06	±	0.56	(3)
Photoheptachlor epoxide	5.28	±	0.59	(3)
9-keto Endrin	11.70	±	1.80	(3)
Telodrin	12.00	±	3.65	(4)
30	13.12	±	1.03	(3)
Endrin	17.03	±	3.81	(3)
Photooxychlordane	29.15	±	3.79	(3)
Isodrin	29.33	±	5.90	(5)
Oxychlordane	34.38	±	3.38	(5)
Photo alpha-Chlordane	46.62	±	3.61	(3)
Dihydroisodrin	50.16	±	3.73	(4)
28	58.45	±	8.72	(3)
Heptachlor-epoxide	66.35	±	4.63	(6)
29	123.75	±	10.64	(3)
Dihydroaldrin	151.63	±	20.64	(3)
Lindane	185.28	±	35.66	(3)
Photochlordene	199.93	±	47.25	(3)
Dieldrin	221.90	±	4.08	(4)
Gamma-Chlordane	289.81	±	27.66	(3)
2,3-Chlordene epoxide	301.69	±	47.05	(4)
Aldrin	342.75	±	33.94	(3)
Heptachlor	350.58	±	50.88	(5)
27	425.80	±	90.44	(3)
26	587.25	±	65.64	(4)
Dihydrochlordene	1759.27	±	132.77	(3)
Alpha-Chlordane	2367.36	±	473.08	(3)
Chlordene	2540.45	±	294.27	(4)
1-Hydroxychlordene	5320.11	±	314.70	(3)
Aldrin cis-diol	9271.47	±	184.66	(3)
Aldrin trans-diol	13320.63	±	486.84	(3)
Chlordecone	18475.38	±	1385.76	(3)
Mirex	No Inhibition			

[1]IC_{50} Values Expressed as Mean ± S.E.

Fig. 1: Binding of ^{35}S-TBPS to rat brain membranes.



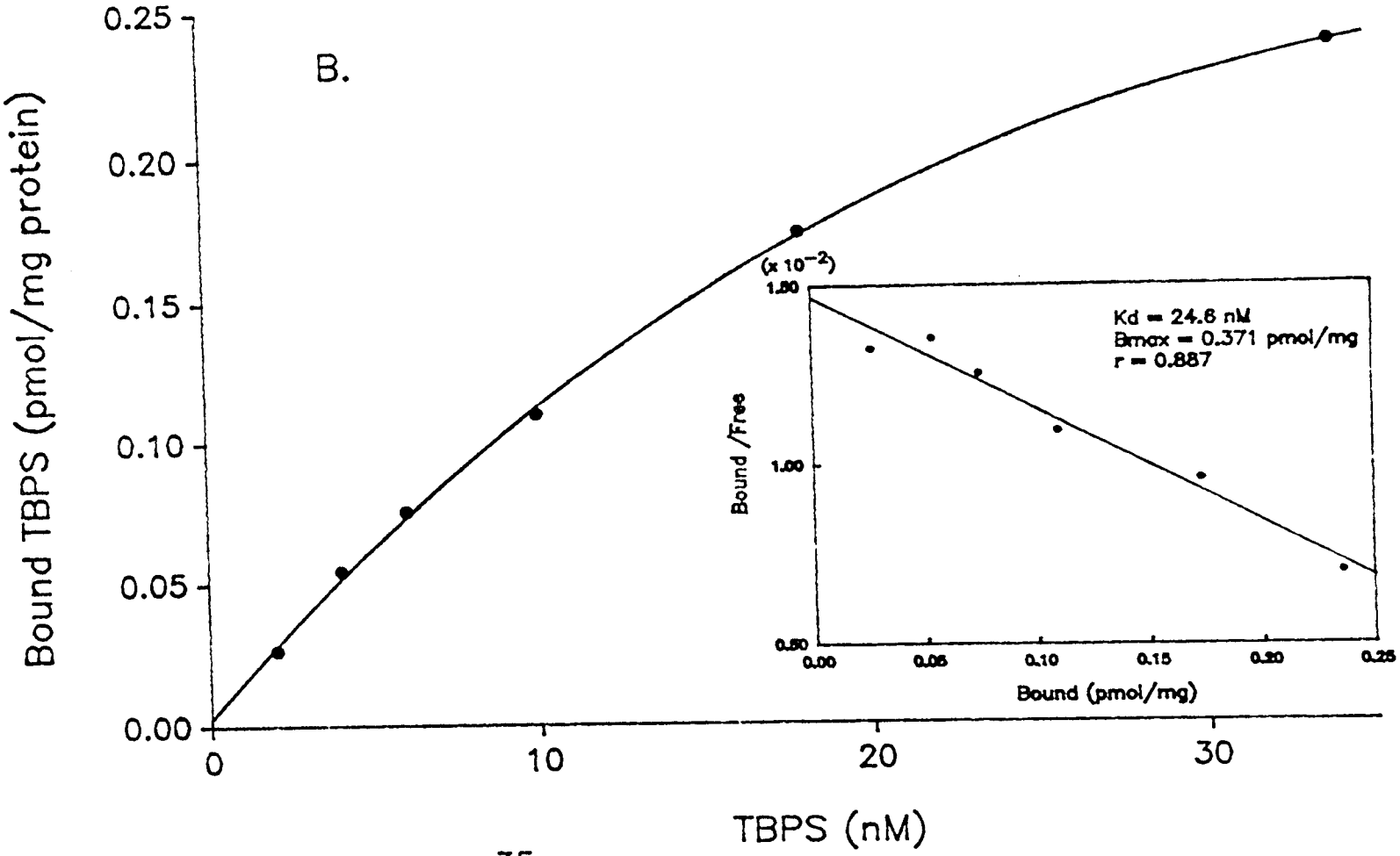
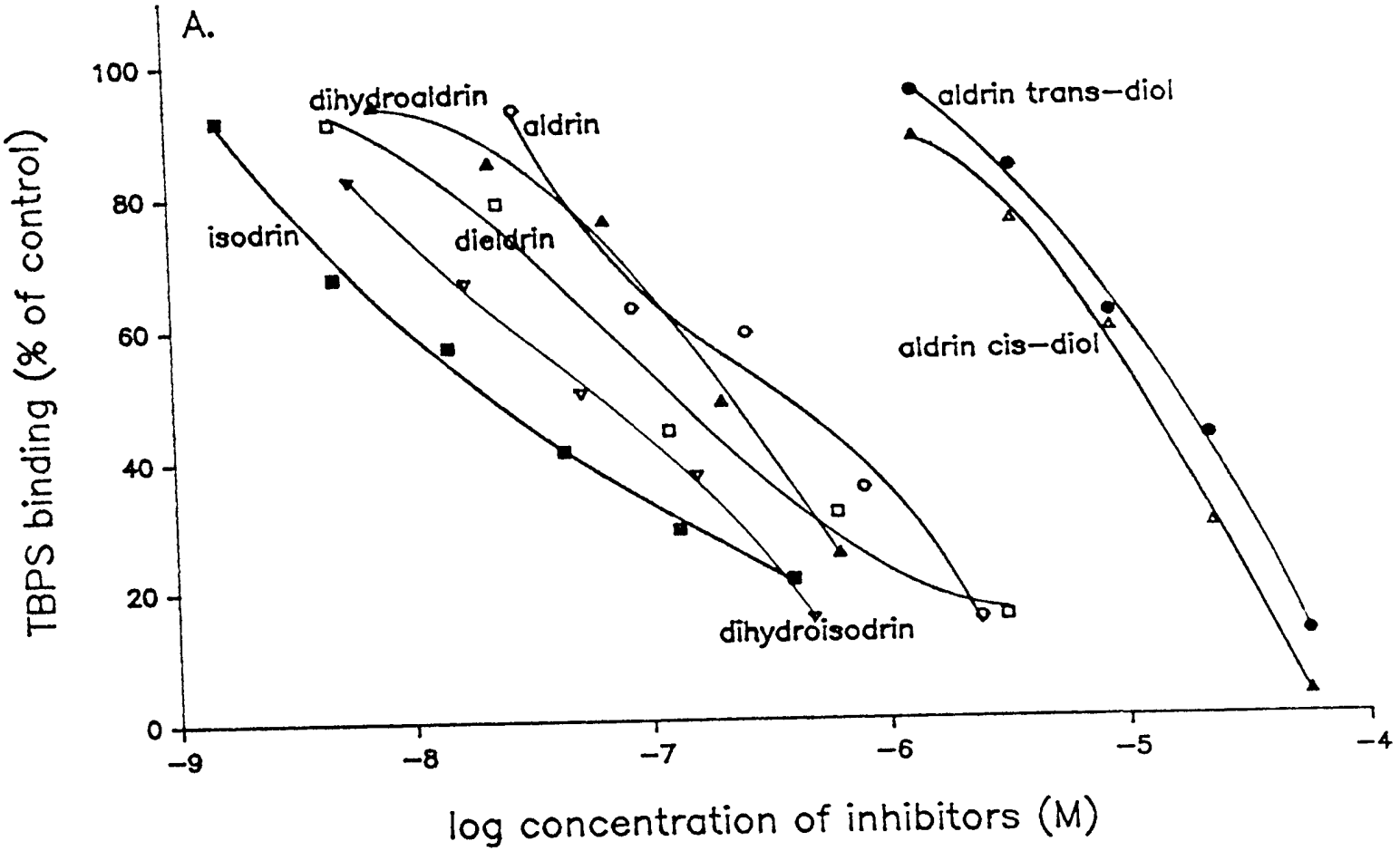
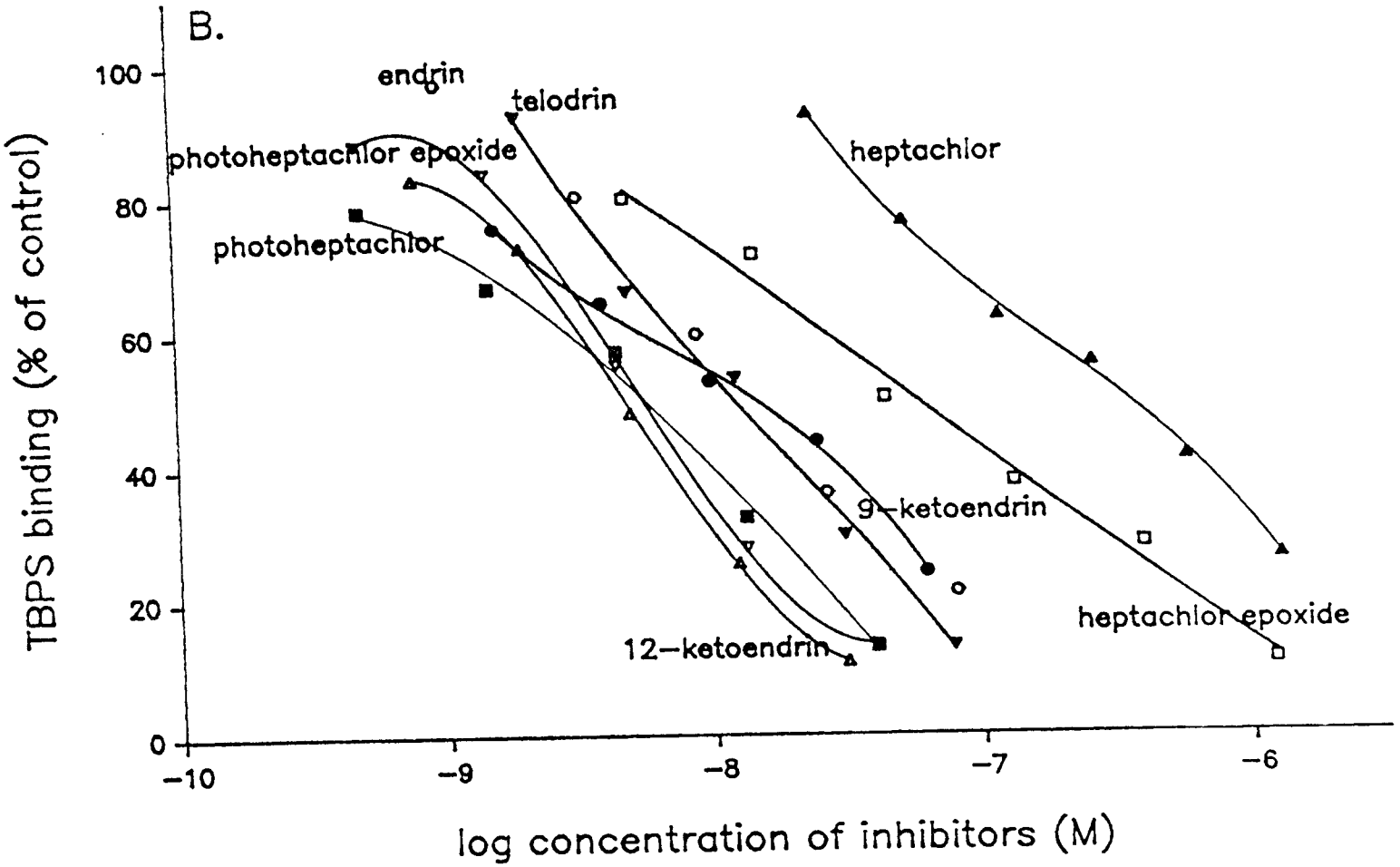
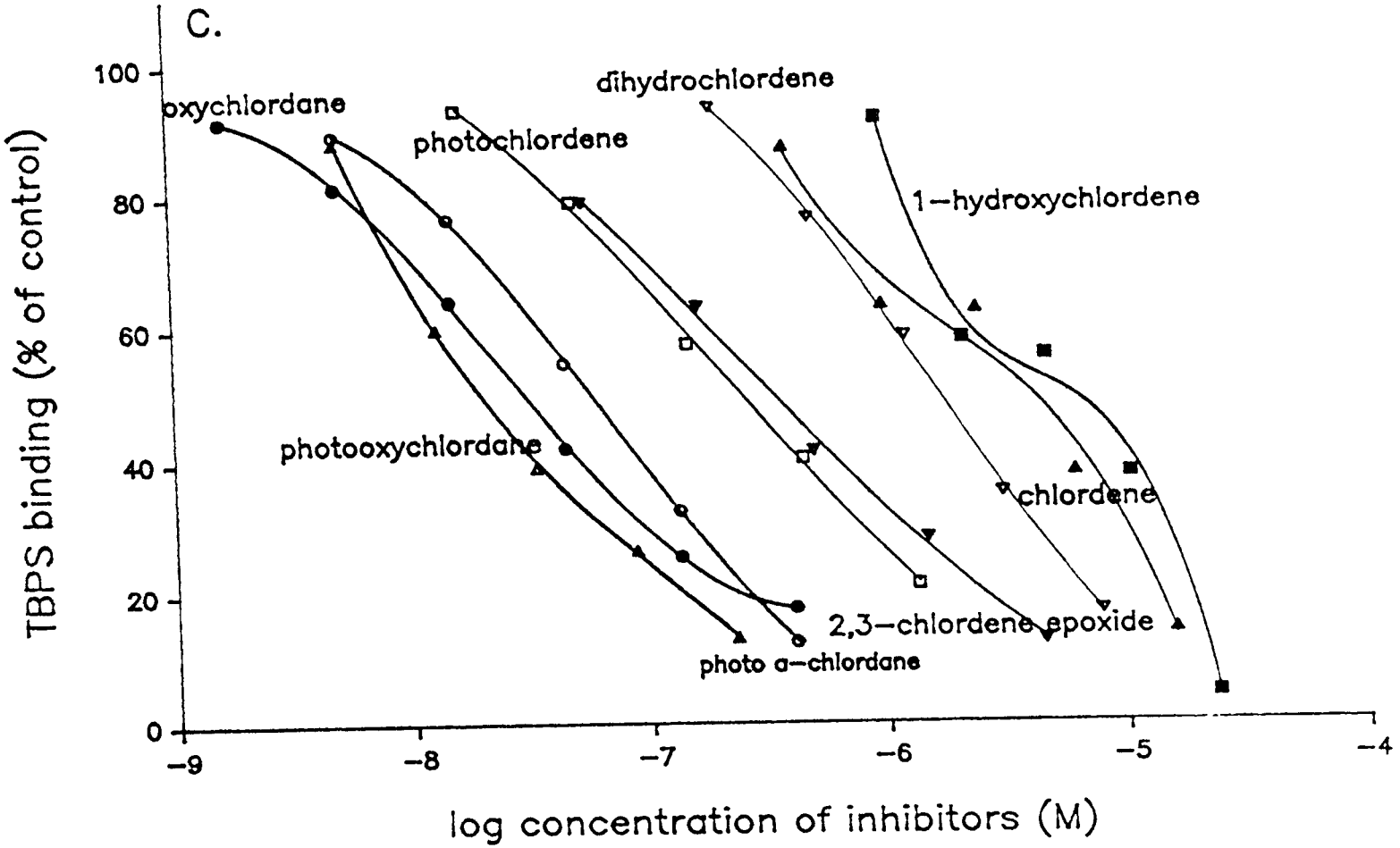


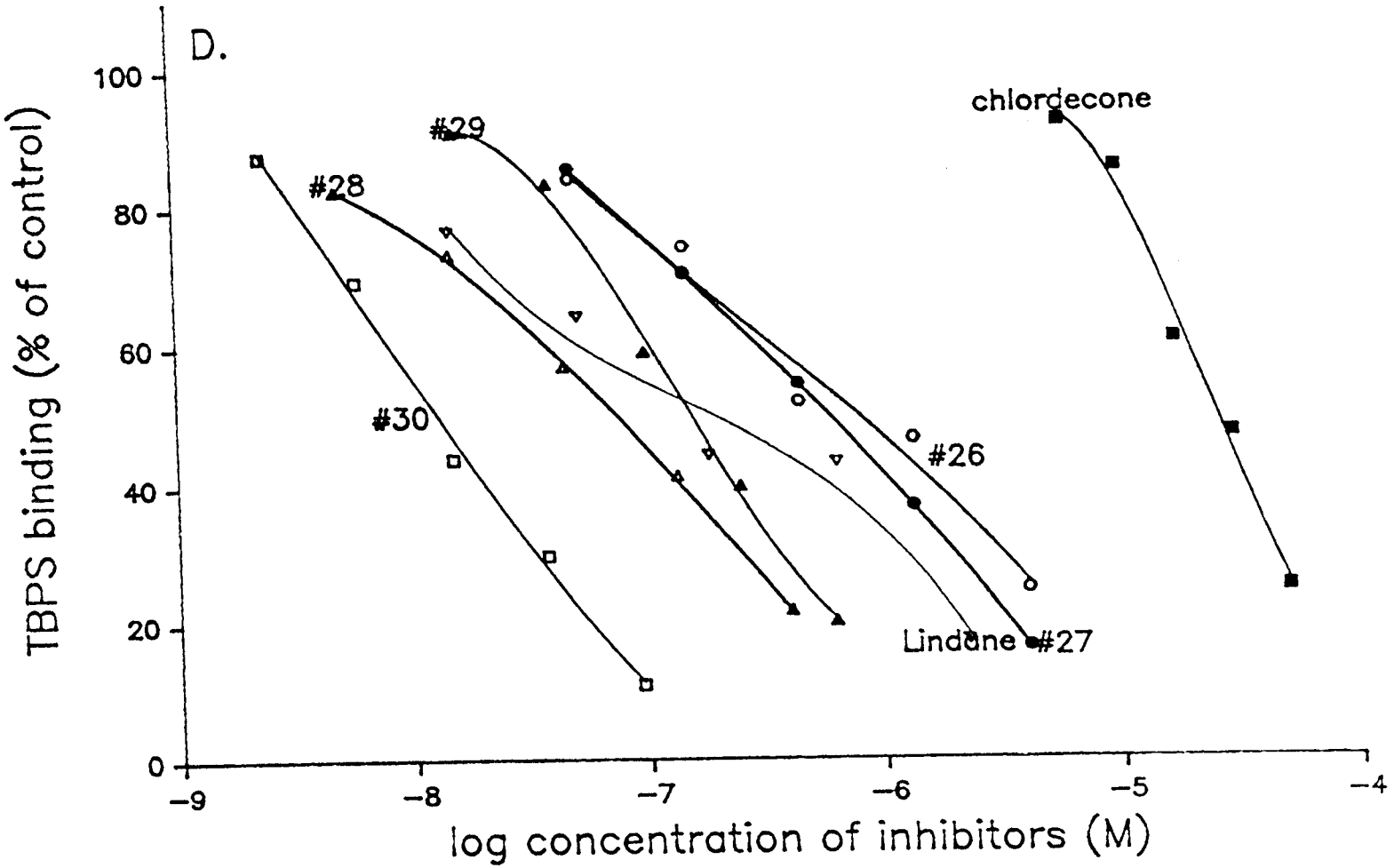
Fig. 2. Specific binding of [^{35}S]TBPS to P_2 membranes from rat brain.
A: as a function of P_2 membrane concentrations.
B: a saturation curve; Inset show Scatchart transformation of the data.

Fig. 3, A-D. Competition of chlorinated alicyclic compounds for ^{35}S-TBPS binding to rat membranes.




B.
endrin
telodrin
photoheptachlor epoxide
heptachlor
photoheptachlor
9–ketoendrin
heptachlor epoxide
12–ketoendrin
100
80
60
40
20
0
−10
−9
−8
−7
−6
TBPS binding (% of control)
log concentration of inhibitors (M)


C.
100
80
60
40
20
0
TBPS binding (% of control)
oxychlordane
photochlordene
dihydrochlordene
1—hydroxychlordene
photooxychlordane
chlordene
2,3—chlordene epoxide
photo α—chlordane
−9
−8
−7
−6
−5
−4
log concentration of inhibitors (M)


D.
TBPS binding (% of control)
100
80
60
40
20
0
−9
−8
−7
−6
−5
−4
log concentration of inhibitors (M)
#28
#29
#30
#26
#27
chlordecone
Lindane

#26: 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,8a-hexahydro-1,4-methanonaphalene.
#27: 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,8,8a-octahydro-1,4,-methanonaphalene.
#28: 5,6,7,8,9,9-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-ethano-5,8-methanonaphalene.
#29: 5,6,7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-2,3-epoxy-1,4-thano-5,8-methanonaphalene.
#30: 5,6,7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-1,4-ethano-5,8-methanonaphalene.

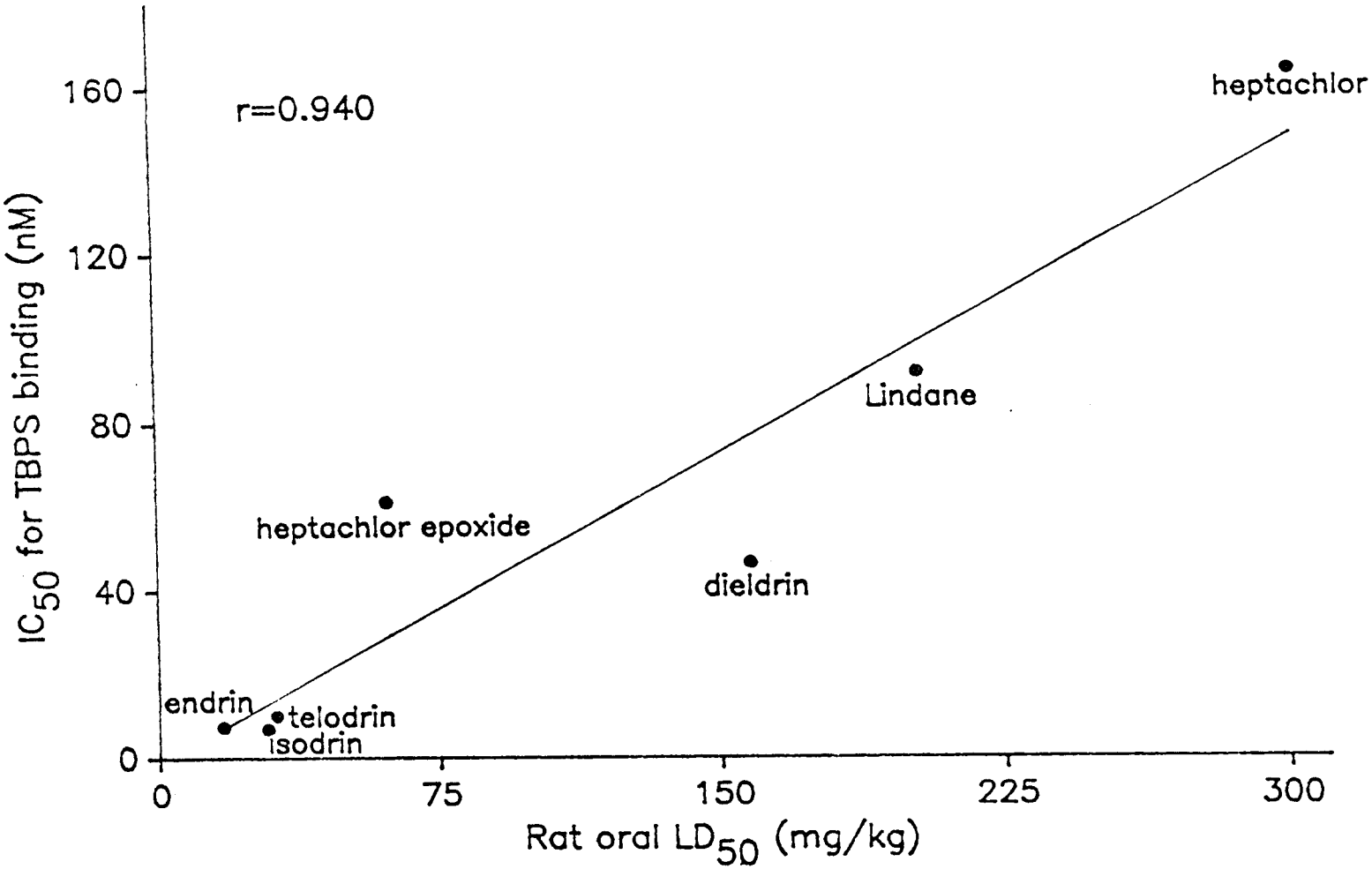


Fig. 4. Correlation by least squares linear regression of inhibitory potency in TBPS binding assays with acute oral toxicity in female rats for chlorinated alicyclic compounds.

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(9), 1995-2004 (1994)

DETERMINATION OF LIPOPHILICITY OF CHLORINATED ALICYCLIC COMPOUNDS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A RP-HPLC procedure has been developed for measuring the capacity factor (k') of a series of chlorinated alicyclic compounds. The chromatographic behavior measured on a 4.5 mm i.d. x 3.3 cm C-18 column with methanol / water as the mobile phase was related to the volume fraction of methanol (ϕ). A linear relationship was found between log k' and ϕ, showing the correlation coefficient γ) 0.99, for each of the 15 chlorinated alicyclic compounds tested. The log k_w , the capacity factor obtained by extrapolation of the retention data from binary eluents to 100 % water, was chosen as a measure of the solute lipophilicity. Since log k_w is considered as a valuble index of the lipophilicity of a compound, the determined values will be used for the quantitative structure-activity relationship studies of the chlorinated alicyclic compounds.

1995

INTRODUCTION

The lipophilicity of a bioactive molecule is one of the most important physicochemical properties which influences its capacity to move through biological compartments. It is generally defined as the tendency of a chemical to distribute between an immiscible nonpolar solvent and water. The logarithm of the partition coefficient of a chemical in the *n*-octanol/water system (log K_{ow}), which is usually measured by 'shake-flask' method, is widely used because of its simplicity and some similarity between *n*-octanol and biological membranes. The 'shake-flask' method works in most cases, but it results in large errors for chemicals with log K_{ow}'s larger than 4, and it is time-consuming and requires considerable amounts of pure stable compounds [1]. It has been proven that the retention capacity factor (k') of a compound in a reversed-phase high performance liquid chromatography (RP-HPLC) system is a reliable indirect descriptor of the lipophilicity of a compound [1-5, 10-16]. Moreover, the recent studies have shown that log k_w, the retention capacity factor which is extrapolated from a binary phase to 100% water in a RP-HPLC system, is an even better descriptor of lipophilicity than the isocratic factor [4-5,10-11].

The chlorinated alicyclic compounds, which were widely insecticides used in the past including aldrin, dieldrin, heptachlor and their structural analogs, constitute a large group of compounds which are environmentally and toxicologically important [6-7]. Their neurochemical action occurs through their binding to the γ-aminobutyric acid (GABA) receptor at the chloride channel [6]. These compounds are generally very non-polar; for example, aldrin has a log K_{ow} as high as 5.9 [9]. But unfortunately, few log K_{ow} 's have been documented for this class of compounds. Direct measurement of their *n*-octanol/water partition coefficients by the conventional 'shake-flask' method is difficult because of their highly lipophilic chracteristics and the availability of adequate amounts for the measurement. In this paper, we are reporting a

systematic study of the lipophilicity of these compounds by using a RP-HPLC method, and the measured data will be used in the on-going reseach of quantitative structure-activity relationships (QSAR) for the compounds.

MATERIAL AND METHODS

Chemicals. The purity for each of the 15 chlorinated alicyclic compounds is greater than 98%. The structures of the compounds were further confirmed by proton-NMR spectra. A stock solution of each compound was made at a concentration of 1mg/ml in methanol and stored at -20 °C. All other chemicals and solvents were of analytical reagent or of HPLC grade.

Apparatus and Chromatographic Conditions. The RP-HPLC system consisted of a Waters 6000A pump coupled with a U-6K injector, a 4.5 mm i.d. x 3.3 cm C-18 analytical column with a particle size of 3 microns, which was manufactured by Perkin-Elmer Corp., Norwalk, Connecticut, a variable-wave-length ultraviolet detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, New Jersey), which was set at 210 nm or 220 nm, and a recorder (Cole-Parmer Instrument Company, Chicago, Illinois).

Measurement of log k'. The dead volume of the system was measured by injecting a 10% $NaNO_3$ solution. The stock solutions of the tested compounds were diluted with methanol to the final injection concentration around 100 μg/ml. A 15-μl injection was made in triplicate. According to their chromatographic behavior, the retention times were determined at five different methanol/water eluent ranges from 60% to 80% of methanol by volume. At each mobile phase composition, the capacity factor was calculated according to $k' = (t_R - t_0)/t_0$, where t_R and t_0 were the retention times of the analyte and of the non-retained compounds respectively. The log k_w values, were obtained

from y-intercept of the plots of log k' *versus* volume fraction of methanol in the mobile phase.

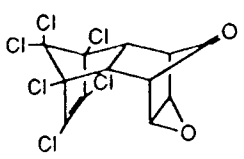
RESULTS AND DISCUSSIONS

The structural information of the 15 tested compounds is given in Figure. 1.

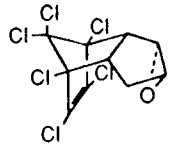
The chromatographs of all tested compounds were accomplished under a variety of conditions in which the volume fraction of methanol (ϕ) in the mobile phase varied from 0.60 to 0.80, since smaller fractions of this component led to unreliable and long retention times. Most of the chlorinated alicyclic compounds are very hydrophobic. This attribute results in unreliably long retention times and trailing of separations by using the commonly used C-18 columns (10 cm or 25 cm in length). However these obstacles were eliminated by introducing a 4.5 mm i.d. x 3.3 cm C-18 analytical column packed with 3-micron support, and this allowed the tested compounds to be eluted at a reasonable time even in the case of the most polar mobile phase. Separations were improved by adding a trace amount of phosphoric acid at a concentration of 0.01% by volume to the mobile phases, and the reproducibility of retention behavior was not affected for the tested compounds (see Figure 2.).

Retention capacity factors (k') at each methanol fraction are given in Table 1 for the 15 tested compounds. Although the monocratic log k's are possibly correlated to other lipophilic descriptors, the established log k' - lipophilicity correlation for a given class of compounds cannot be extrapolated either to different solutes or to other similar or even identical separation systems, and it may result in misleading data owing to solute-solvent interactions [4].

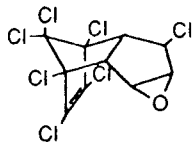
The log k_w, the retention capacity factor of a compound when 100% water is employed as mobile phase in a RP-HPLC system, was used for evaluating the lipophilicity of the compounds because it eliminated



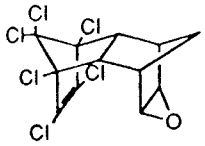
1. 12-Ketoendrin



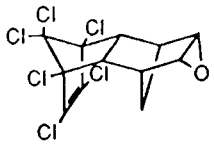
2. Chlordene epoxide



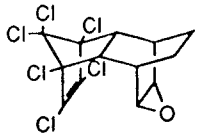
3. Heptachlor epoxide



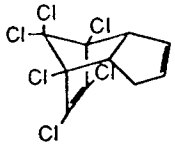
4. Endrin



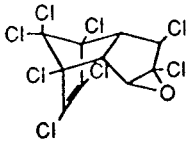
5. Dieldrin



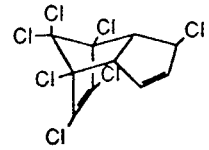
6. Epoxide of 14



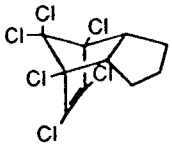
7. Chlordene



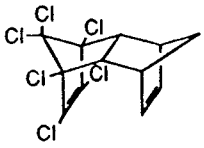
8. Oxychlordane



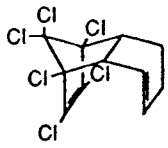
9. Heptachlor



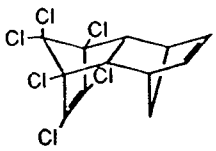
10. Dihydrochlordene



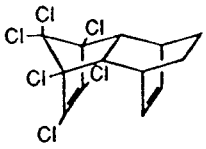
11. Isodrin



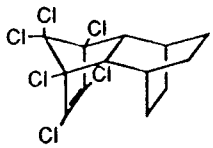
12. @



13. Aldrin



14. @@



15. Octahydro-derivative of 14

FIGURE 1. Structures of the Tested Compounds

@: Hexachlorocyclopentadiene/cyclohexa-1,3-diene adduct
@@: Hexachloronorbornadiene/cyclohexa-1,3-diene adduct

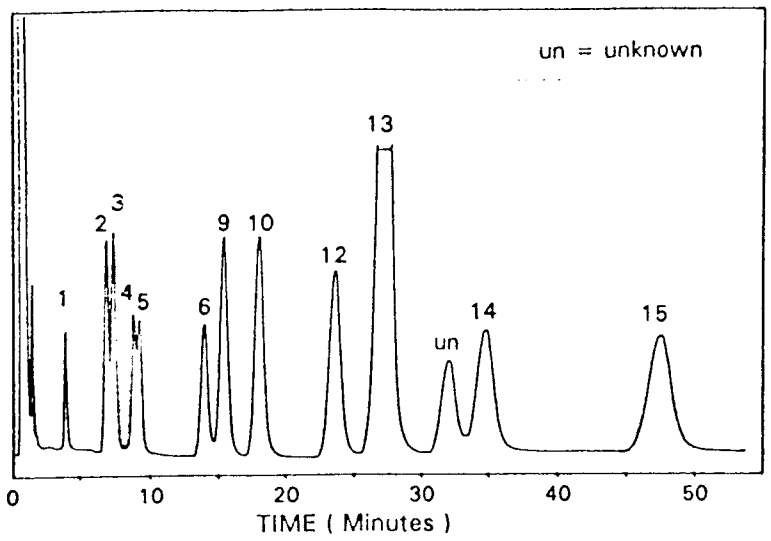


FIGURE 2. HPLC Profile of the Tested Compounds. Column: 4.5 mm i.d. x 3.3 cm C-18 cartridge pack; Mobile phase: 70/30 methanol/water + 0.01% H_3PO_4; Flow rate: 1.0 ml/min; Detector: UV-210 nm; Temp.: 25 °C.

selective solute-solvent interactions and is more closely related to log K_{ow} than isocratic capacity factors [4, 12-16].The log k_w' was determined by extrapolating the polycratic retention capacity factors (log k's) from binary eluents to 100% water. It was found that for the 15 tested compounds, the relationship between solute retention and the composition of methanol in the mobile phase can be described by the equation:

$$\log k' = \log k_w - S\phi \quad (1)$$

where S refers to the slope of log k' *vs.* ϕ plots. The corrlation coefficients ($\gamma > 0.99$) showed that log k' and ϕ were highly linearly correlated for the 15 compounds. The slopes for the equations were in the scope of 4.84 to 6.93. The extrapolated log k_w's are given in Table 2.

TABLE. 1 Isocratic Retention Capacity Factors (k') of the Tested Compound

Compound	ϕ: Methanol/water (v/v)				
Number	0.60	0.65	0.70	0.75	0.80
1	22.737	11.562	7.342	3.991	2.388
2	46.286	23.366	14.081	6.887	4.253
3	53.571	26.158	15.211	7.695	4.141
4	63.395	30.921	18.363	9.296	5.285
5	71.150	33.406	19.443	9.620	5.252
6	112.514	52.522	30.467	14.946	8.019
7	120.974	54.229	28.844	14.345	7.594
8	136.601	59.478	30.662	14.495	7.215
9	136.601	60.777	33.650	14.956	7.722
10	161.867	71.660	39.641	18.238	10.103
11	220.067	87.790	50.001	20.690	11.278
12	222.032	96.616	52.047	21.391	12.224
13	267.860	113.063	60.476	24.074	13.832
14	357.519	149.764	78.426	29.466	17.502
15	517.449	210.398	108.462	43.807	20.961

TABLE 2. Linear Relationship between Log k' and ϕ:
$\log k' = \log k_w - S\,\phi$

Compound	S	$\log k_w$	γ^2 (n = 5)*
1	4.84	4.24	0.9970
2	5.21	4.78	0.9965
3	5.51	5.02	0.9987
4	5.36	5.00	0.9979
5	5.61	5.20	0.9980
6	5.68	5.44	0.9980
7	5.96	5.64	0.9983
8	6.34	5.92	0.9989
9	6.21	5.85	0.9980
10	6.01	5.79	0.9971
11	6.42	6.16	0.9945
12	6.35	6.14	0.9956
13	6.49	6.30	0.9948
14	6.70	6.55	0.9939
15	6.93	6.86	0.9989

* γ = Correlation coefficient.

The relationship between the slope S and the intercept values (log k_w) was investigated for the tested compounds. A good linear correlation was observed: correlation coefficient = 0.9937. Slope S depends on the size of the solute molecule and the structure of polar functional groups. The high linear correlation coefficient may be a reflection of the uniqueness and suitability of the methanol-water system for estimating the lipophilicity of the compounds [4].

CONCLUSION

The retention capacity factor (log k') of a compound in a RP-HPLC system can be used as a descriptor of its lipophilicity. The isocratic log k' was measured at five different compositions of the eluent, and the log k_w was extrapolated from the linear relationship between log k' and the fraction of methanol in the mobile phase for each of the 15 chlorinated alicyclic compounds. The log k_w's may be advantageous in describing the lipophilic properties of the structurally related, very nonpolar chlorinated alicyclic compounds, which for use in QSAR studies.

ACKNOWLEDGEMENTS

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REFERENCES

1. Van de Waterbeemd, H. and Testa, B., The parameterization of lipophilicity and other structural properties in drug design. Adv. Drug Res. 16: 85, 1987.

2. Ellgehasen, H., D'hondt, C. and Fruerer, R., Reversed-phase chromatography as a general method for determining octan-1-ol / water partition coefficient. Pestic. Sci. 12: 219, 1981.

3. Garst, J. E. and Wilson, W. C., An accurate, wide-range, automated, high-performance liquid chromatographic method for the estimation of octanol/water partition coefficient. I. Effect of chromatographic conditions and procedure variables on accuracy and reproducibility of the method. J. Pharm. Sci. 73: 1616,1984.

4. Braumann, T., Determination of hydrophobic parameters reversed-phase liquid chromatography: theory, experimental techniques, and application in the studies on quantitative structure-activity relationships. J. Chromatogr. 373:191, 1986.

5. Calvino, R., Fruttero, R. and Gasco, A., Reversed-phase high-performance liquid chromatographic study of the lipophilicity of a series of analogues of the antibiotic "calvatic acid". J. Chromatogr. 547: 167, 1991.

6. Lawrence, L. J., and Casida, J. E. Stereospecific action of pyrethroid insecticides on the γ-aminobutyric acid receptor-ionophore complex. Science 221: 1399-1401, 1983.

7. Coats, J. R., Mechanism of Toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. Environ. Health Perspect. 87: 255, 1990.

8. Hartley, D., and Kidd, H. (Ed.), The Agrochemicals Handbook, The Royal Society of Chemistry, Nottingham, England, 1989.

9. Kenaga, E. E. and Goring, C. I. A., Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration of chemicals in biota. Aquatic Toxicol., ASTM STP 707, Eaton, J. G., Parrish, P. R., and Hendricks, A. C., eds. American Society for Testing Materials, Philadephia, pp78-115, 1980.

10. Demotes-Mainard, F., Jarry, C., Thomas, and Dallet, P., RP-HPLC retention data of new 2-amino-2-oxazolines. An approach of their lipophilic properties. J. Liquid Chromatogr. 14 (4): 795, 1991.

11. Pereira, A. L., Barreiro, E. J. L., Freitas, A. C. C., Correa, C. J. C., and Gomes, L. N. L. F., Investigation of the lipophilicity of antiphlogistic pyrazole derivatives: Relationships between log Kw

and Log P values of 5-arylamino and arylhydrazono-3-methyl-4-nitro-1-phenylpyrazoles. J. Liquid Chromatogr. 14 (6): 1161, 1991.

12. Hammers, W. E., Meurs, G. J., and de Ligny, C. L., Correlation between liquid chromatographic capacity ratio data on Lichrosorb RP-18 and partition coefficient in the octanol-water system. J. Chromatogr. 247: 1, 1982.

13. El Tayar, N., Van de Waterbeemd, H. and Testa, B., The prediction of substituent interactions in the lipophilicity of disubstituted benzenes using RP-HPLC. Quant. Struc.-Act. Relat. 4: 69, 1985.

14. Harnisch, M., Möckel, H. J. and Schulze, G., Relationship between log P_{ow} shake-flask values and capacity factors derived from revesed-phase high performance liquid chromatography for n-alkylbenzenes and some OECD references. J. Chromatogr. 282: 315, 1983.

15. Thus, J. I. G. and Kraak, J. C., Comparision of phenyl- and octadecyl-modified silica gel as stationary phase for the prediction of n-octanol-water partition coefficient by high-performance liquid chromatography. J. chromatogr. 320: 271, 1985.

16. Butte, W., Fooken, C., Klussmann, R. and Schuller, D., Evaluation of lipophilic properties for a series of phenols, using reversed-phase high performance liquid chromatography ang high performance thin layer chromatography. J. Chromatogr. 214: 59, 1981.

17. Murakami, F., Retention behavior of benzene derivatives on bonded revesed-phase columns. J. Chromatogr. 178: 393, 1979.

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Quantitative Structure-Activity Relationships (QSAR) of Chlorinated Alicyclic Compounds - Chemical Assays and Analysis

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III. QSAR and Current Research Status

Quantitative Structure-activity Relationships (QSAR)

The study of 'structure and action' usually in particular concerns 'quantitative structure-activity relationships' (QSAR) among a group of bioactive agents, which in their molecular mechanism of action, the sites of action, receptor, enzymes, carriers, etc. are analogs. This implies that the group of compounds compared in the study can be assumed to bind to largely overlapping domains on their molecular sites of action. This in turn means that the study should be restricted to congeneric series of compounds, which implies a 3-dimensional homology of various molecular fragments in the chemical framework of the series.

QSAR should comprise structure-activity and -selectivity relationships. There is always a comparison of compounds. In practice, families of compounds modulated by particular regional structural changes, substitutions, etc., are considered. This is particularly clear in QSAR based on the contributions of regionally restricted substituents in the regression introduced by Hansch and the various related approaches. If the correlation in the family of compounds studied is poor, this may indicate poor experimentation, poor statistics, or , if these are excluded, a poor congenerity. In that case outliers with an appreciable activity may serve as potential leads for other families of congeneric compounds. In some cases, on the basis of the comparison of the chemical structure, a cloud of experimental data may generate different regression lines indicating the involvement of non-congeneric families of compounds differing for instance in accessory binding domains, but still involved in a common receptor site on common receptors. The selection of compounds for QSAR is based on a presumed congenerity. In fact the results of proper QSAR reveal how far true congenerity is involved.

The mechanism of action of some chlorinated cyclodienes has been purported to involve binding at the picrotoxinin site in the γ-aminobutyric chloride ionophore complex. Several of those chlorinated insecticides have been shown to bind at the picrotoxinin site at which *t*-butylbicyclophosphorothionate (TBPS) also binds. Thus competition of chlorinated cyclodienes for radiolabelled TBPS binding can be used as an endpoint to express their activity, i.e., the median inhibitory concentration (IC_{50}).

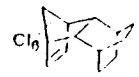
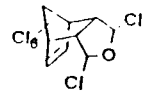
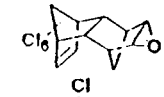
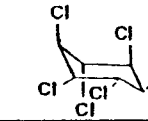
Compounds originally proposed in this study include 33 chlorinated alicyclics. Most of them are compounds produced by the Diels-Alder diene reaction, and they are closely related structures. However, further classification of these compounds is logical based their congenerity. Compounds with the chlordene nucleus may constitute a closely structurally related family as do the group of aldrin-like compounds. Photorearrangement products of these compounds may have special 3-dimensional conformation, and structural rigidity. Obviously, lindane is less congeneric to the above compounds. And possibly chlordecone and mirex may also contain specific structural attributes different from the above chlordene type and aldrin type families.

The goal of QSAR is an optimization of the relationship between the dose and the observed biological response, predicting the biological activity of compounds, even though the compound may not, as yet, exist, and probing the characteristics of ligand-receptor interactions for compounds for which the mechanism of action is not fully understood yet.

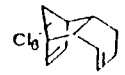
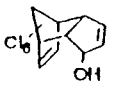
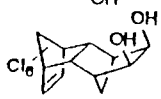
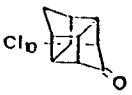
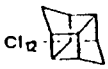
Lipophilicity and QSAR

Lipophilicity plays an important role in QSAR in two different ways. There is the 'substituent contribution', the regional contribution to lipophilicity involved in the interaction of compounds with its specific binding domains, and there is the 'integral lipophilicity'

Table 1. Inhibition of specific [^{35}S]-TBPS binding to rat brain P2 membranes by chlorinated alicyclic compounds (IC_{50}).

No.	Compound		IC_{50} (nM) n=3 or 4	CV@ (%)	Log(1/IC_{50})
1	12-ketoendrin		4.22 ± 0.42	10.0	-0.625
2	Photoheptachlor epoxide		5.26 ± 0.82	15.6	-0.721
3	9-ketoendrin		11.6 ± 2.40	20.7	-1.065
4	A*		13.1 ± 1.47	11.2	-1.117
5	Endrin		16.9 ± 5.10	30.2	-1.228
6	Isodrin		28.9 ± 5.40	18.7	-1.461
7	Photooxychlordane		29.2 ± 5.30	18.2	-1.465
8	Telodrin		31.2 ± 18.3	58.7	-1.494
9	Oxychlordane		36.6 ± 20.1	54.9	-1.564
10	Dihydroisodrin		48.0 ± 7.2	15.0	-1.681
11	B*		56.6 ± 13	23.0	-1.753
12	Heptachlor epoxide		61.4 ± 29	47.4	-1.788
13	Dihydroaldrin		140 ± 60	42.9	-2.146
14	C*		145 ± 44	30.3	-2.161
15	Dieldrin		158 ± 51	32.3	-2.199
16	Lindane		203 ± 41	20.2	-2.308

(Table 1 continued)

17	Photochlordene		210 ± 71	33.8	-2.322
18	Heptachlor		302 ± 99.3	32.9	-2.480
19	Aldrin		319 ± 42	13.2	-2.504
20	2,3-chlordene epoxide		334 ± 70	21.0	-2.524
21	D*		681 ± 253	37.2	-2.833
22	E*		737 ± 294	39.9	-2.868
23	Dihydrochlordene		1,660 ± 197	11.9	-3.220
24	Chlordene		2,577 ± 484	18.8	-3.411
25	1-hydroxychlordene		4,449 ± 538	12.1	-3.648
26	Aldrin *cis*-diol		8,778 ± 886	10.1	-3.943
27	Aldrin *trans*-diol		13,564 ± 1,163	8.6	-4.133
28	Chlordecone		22,734 ± 6,674	29.4	-4.357
29	Mirex		"No inhibition"	-	-

@: Coefficient of variation.

*: A, 5,6,7,8,9,9,-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-1,4-ethano-5,8-methanonaphthalene; B, 5,6,7,8,9,9,-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-ethano-5,8-methanonaphthalene; C, 5,6,7,8,9,9,-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-2,3-epoxy-1,4-ethano-5,8-methanonaphthalene; D, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene; E, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,8a-hexahydro-1,4-methanonaphthalene.

represented by the Σ hydrophobic fragmental constants for the molecule as a whole. The latter is of particular significance for a mechanism based on passive transport, such as that involved in the blood-brain barrier passage, tissue uptake, passive renal reabsorption, etc. The overall lipophilicity apparently also contributes to the binding of drug molecules to receptors, enzymes, plasma proteins, etc. This is not only related to the lipophilicity in particular regions of the molecule critical in its interaction with particular binding domains, but also to the shift of the active molecule as a whole from the free water phase to the surface of biological macromolecules and structures such as membranes.

Log K_w, the lipophilic descriptor used in this study which is parallel to the logarithm of the partition coefficient between 1-octanol and water [1, 2], is clearly a parameter expressing the overall lipophilic characteristics of a molecule. The relationship between Log K_w and the activity on inhibition of TBPS binding (Table 1) is shown in Figure 1. The cloud of the scatter-plot of Log K_w versus the IC_{50}'s may indicate the necessity of further sorting of the compounds according to their structural congenerity.

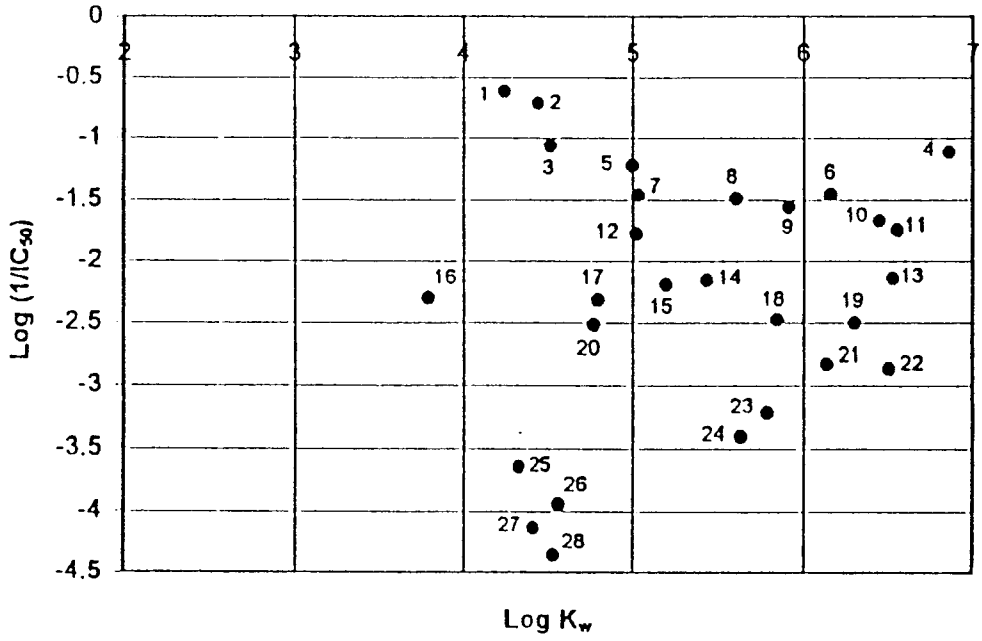


Figure 1. The scatter-plot of IC_{50}s for TBPS binding inhibition vs. lipophilic parameter, log K_w, for 28 chlorinated compounds.

According to their structures, the compounds could be classified into two groups. Compounds in Group I (Figure 2) contain epoxide or ketone functional groups. The relationship shows that the activity as inhibitors of TBPS-binding of the compounds increases as they become more hydrophilic. However, a low coefficient of determination ($R^2 = 0.2516$) did not indicate a good linear relationship (Figure 2A). The linearity was much improved on trimming off oxychlordane and telodrin as outliers (Figure 2B). The data on inhibiting TBPS binding of these two compounds lack reliabilities, and the coefficient of variation were 54.9% and 58.7%, respectively (Table 1).

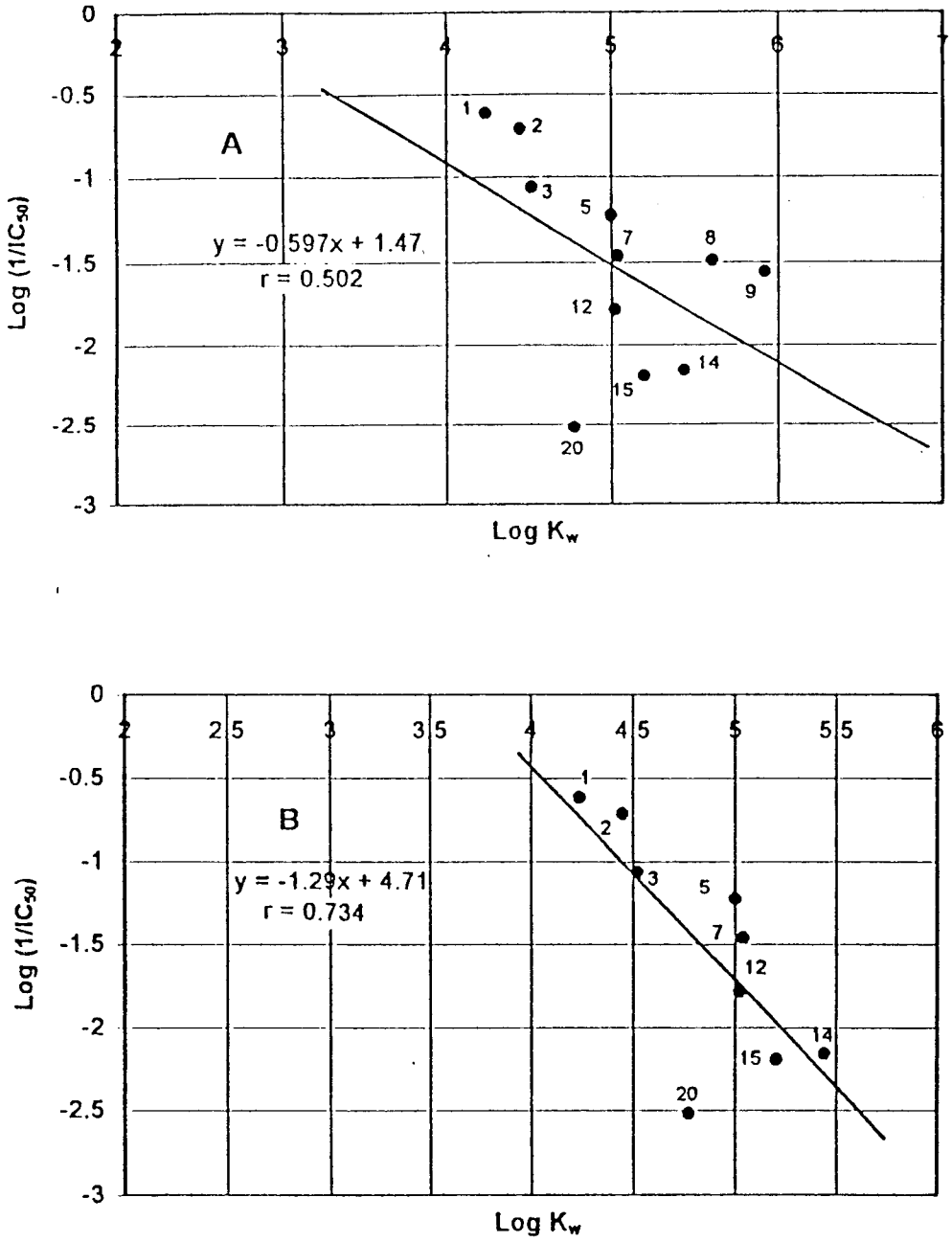


Figure 2. Relationship between Log K_w and IC_{50} for compounds in Group I.
A: Oxychlordane (No. 9) and telodrin (No. 8) were not trimmed.
B: Oxychlordane and telodrin were trimmed as outliers, based on their very high coefficient of variation in the *in vitro* assays (see Table 1).

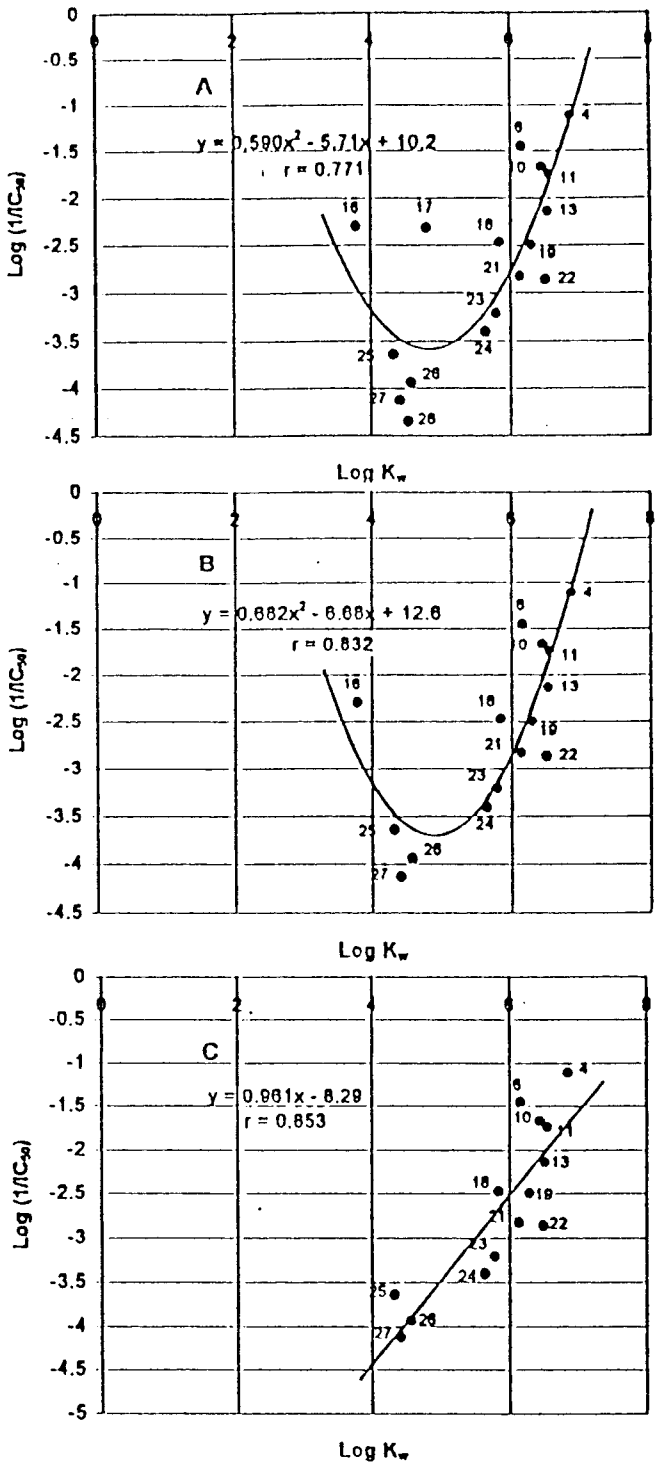


Figure 3. Relationship between Log K_w and IC_{50} for compounds in Group II.
A: Photochlordene (No. 17) and chlordecone (No. 28) were not trimmed.
B: Photochlordene and chlordecone were trimmed as outliers, based on their cage structure rather than cyclodiene structure (see Table 1).
C: Lindane (No. 16) was trimmed as an outlier (see dissimilarity of structure, Table 1), as were No. 17 and No. 28.

Structurally, compounds in Group I bear electronegative atoms, and it is more interesting that these compounds have an epoxide group except 9-ketoendrin. The epoxide is much more potent than its parent form, for example, dieldrin and aldrin, and heptachlor epoxide and heptachlor. In contrast to Group I, the compounds in Group II, showed that their IC_{50}s increase as they become more hydrophobic, and a quadratic relationship exists among them (Figure 3). None of these compounds in Group II bears an epoxide group. Compounds such as aldrin *cis*- and *trans*-diols are among the compounds with the lowest potency, perhaps due to their poor lipophilicity.

The opposite relationships in Group I and II compounds between lipophilicity and activity on inhibition of TBPS binding may indicate specific ligand-receptor interactions, since the activity measurement is an *in vitro* assay in this study. The complicating influences of penetration, metabolism, and excretion were eliminated, and it seems that Group I and Group II compounds might bind in different ways or to different regions respectively on the common binding site. The epoxide form of chlorinated cyclodienes may be an essential feature for eliciting their high inhibitory activity on the GABA receptor. The alkene form of the parent compounds can be converted to the epoxide form by bioactivation metabolism in *in vivo* cases. Relevant information was reported that the coincidence of biotransformation of heptachlor, aldrin, and isodrin to their epoxides in the tissues of the treated insects with the onset of the poisoning symptoms was observed [3]. Compounds in Group II do not bear the epoxide group, and their action may partially be effected by interaction with membranes or a lipophilic region of the GABA receptor. As these compounds become more lipophilic, a stronger interaction may arise to the region, and result in more damage to the integrity or flexibility of the receptor.

Both photochlordene and chlordecone are cage-like compounds, and they may lack congenerity as compared to other compounds in Group II. They were trimmed as outliers when sorting the data. Initially, lindane was not trimmed as an outlier despite its structural dissimilarity to chlorinated cyclodienes, but the quadratic curve is difficult to explain (Figure 3A and 3B). When lindane (No. 16) is trimmed, the resultant series of 14 congeneric cyclodiene structures (without oxygen atom) showed a good correlation between lipophilicity and inhibition of TBPS binding (Figure 3C). Eldefrawi *et al.* reported that lindane also displaces competitively the [^{35}S]-TBPS to bind to a putative voltage-dependent chloride channel of *Torpedo*, and the affinity of lindane for these putative voltage-dependent channels is higher than that for the $GABA_A$ receptor [4]. Mirex has no inhibitory effect on TBPS binding, and chlordecone has the next lowest potency in this study. Although chlordecone inhibits binding of [^{35}S]-TBPS to the putative voltage-dependent chloride channel, mirex does not [4]. Since these two insecticides share some of their toxicity symptoms with lindane, and mirex may be metabolically converted to chlordecone, then it is tempting to speculate that voltage-dependent chloride channels may also play a role in the action of these insecticides. However, current hypotheses seem to indicate that chlordecone and mirex probably do not act primarily at the GABA receptor site, but that lindane does. This current project has shown chlordecone and mirex to very inactive and lindane to be somewhat active.

Molecular connectivity and QSAR

Molecular connectivity indices have been shown to be rich in structural information related to topological, geometric and spatial attributes [5]. Zero- first- and second-order of valence molecular connectivity indices have shown significant linear relationships with the activity on inhibition of TBPS binding for most of the 29 compounds studied, indicating the predictive

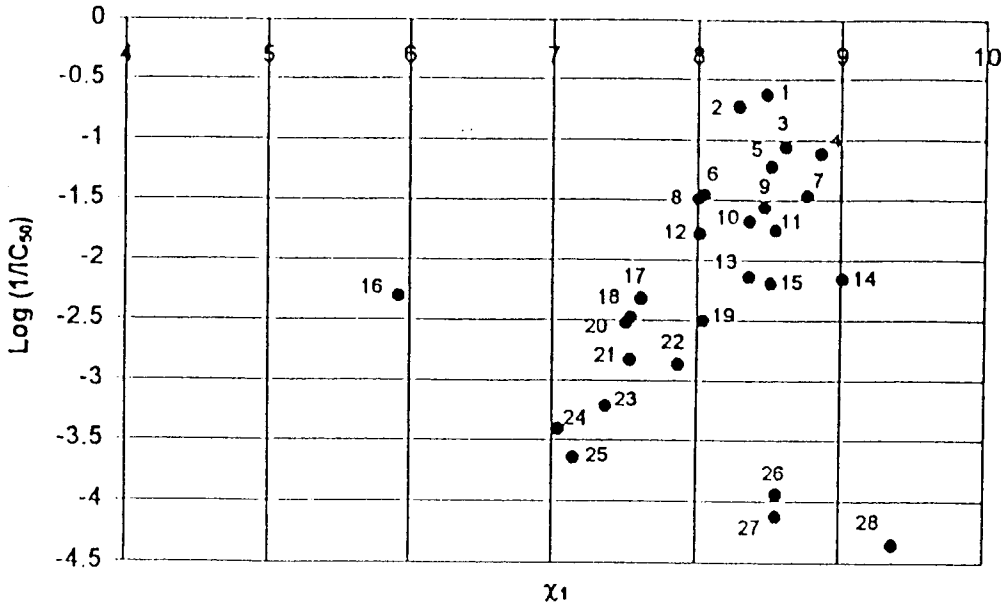


Figure 4. Relationship between first-order of valence molecular connectivity index and IC_{50} for 28 chlorinated alicyclic compounds.

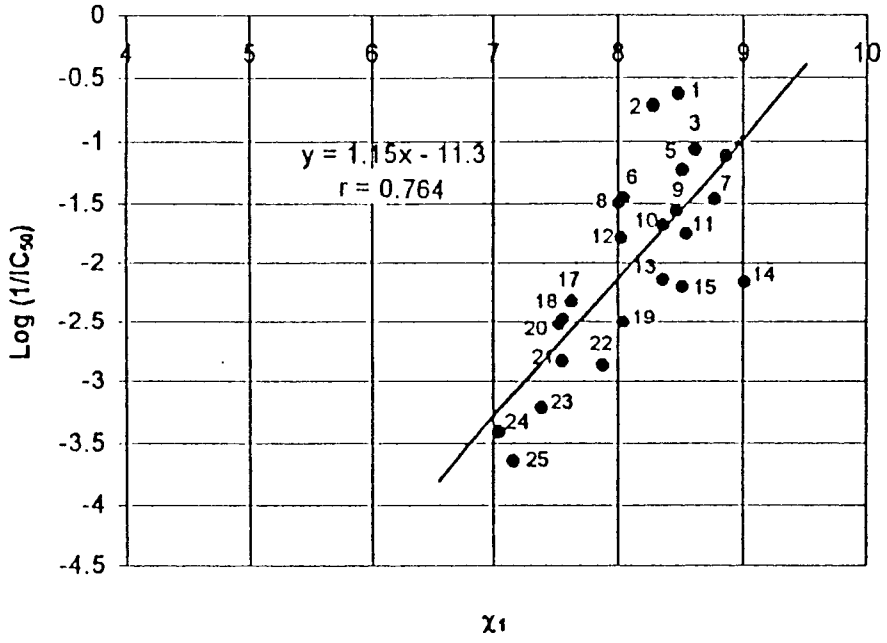


Figure 5. Relationship between first-order of valence molecular connectivity index and IC_{50} after trimming lindane (No. 16), aldrin *cis*-diol (No. 26), aldrin *trans*-diols (No. 27), and chlordecone (No.28), as outliers; lindane is structurally dissimilar, while the two diols and chlordecone have the weakest activities and are dissimilar as well.

power of the molecular connectivity index for the chlorinated alicyclic compounds. A linear relationship is illustrated here (Figures 4 and 5). The first-order valence molecular connectivity index (χ_1) gives weight to structural features of one-bond-length fragments in the molecules. The positive linear relationship indicates that with increasing number of atoms, the inhibitory effect of a compound also increases, and also with an increase in the heteroatom content of a compound, the inhibitory effect increases.

Similar to that mentioned earlier, lindane and chlordecone were trimmed as outliers because they are not congeneric to cyclodiene analogs. Aldrin cis- and trans-diols were also trimmed. The reason for eliminating these two chemicals is that the molecular connectivity index may not be so definitive as to differentiate an ether-like oxygen atom from a hydroxyl oxygen atom for closely structurally related compounds. Also, they have extremely low activity in the in vitro assay, as does chlordecone (see No. 26, 27, and 28 in Figure 4). The three compounds can essentially be considered inactive in binding competitively with TBPS. Again, lindane, a cyclohexane, is dropped from the series of compounds because all other members are cyclodiene congeners.

In summary, the molecular connectivity index correlated well with the compounds' binding at the site of action, if the series is limited to the chlorinated alicyclic compounds of close structural similarity.

Conclusions to date

For lipophilic QSAR determinations, limiting the series to structurally similar analogs and splitting those into two groups (oxygen-containing and non-oxygen-containing) yielded very good correlations. The opposite relationships for the two groups may be a function of different types of lipophilic interactions at the receptor site.

For molecular connectivity, there exist clear relationships between the zero, first, and second order indices and the activity of the compounds in the TBPS-binding assay. Again the series of compounds needed to be restricted to the 24 close structural analogs for the relationship to be of significant statistical strength.

It is hoped that these relationships will be of predictive value if other cyclodiene molecules are discovered with potential as pesticides or drugs. Futher, this research intends to help describe, with a little more quantitative detail, the nature of the GABA receptor-chloride ionophore complex.

Future Work

Both the Log K_w and the molecular connectivity index so far investigated are 'integral descriptors', i.e., considering the molecule as a whole. The substituent contribution, the regional descriptors, which may describe physicochemical properties or topological characteristics of a specific substituent or functional group in a molecule, are not used because of the structural diversity of the compounds under study. Chemical shift data in proton NMR did not show significant relationships with biological activity when we tried to use them as regional electronic descriptors. ^{13}C-NMR may be used as a regional electronic parameter at the atom level because it is more sensitive to the chemical environment changes within a molecule based on the sorting of the compounds into more structurally related groups. Other descriptors, such as the electrotopological state index developed by Kier and Hall [6], which encodes information about both the topological environment of a specific atom and the electronic interactions due to other atoms in the molecule, may also be applicable to the QSAR study.

Selected References

1. Liu, J., Chambers, J. E., and Coats, J. R., 1994. Determination of Lipophilicity of Chlorinated Alicyclic Compounds by Reversed-phase High Performance Liquid Chromatography. J. Liquid Chromatogr. 17(9): 1995-2004.
2. Braumann, T., 1986. Determination of Hydrophobic Parameters by Reversed-phase Liquid Chromatography: Theory, Experimental Techniques, and Application in the Studies on Structure-Activity Relationships. J. Chromatogr. 373: 191.
3. Yawetz, A. and Tahori, A. S., 1993. In memoriam: Albert S. Perry. Archives of Insect Biochemistry and Physiology 22:1-3.
4. Eldefrawi, M. E., Gant, D. B., Abalis, I. M., and Eldefrawi, A. T., 1987. Interactions of Insecticides with GABA-gated and Voltage-dependent Chloride Channels in *Sites of Action for Neurotoxic Pesticides* (Hollingworth and Green ed.), page 107-121, American Chemical Society, Washington, DC.
5. Kier, L. B. and Hall, L. H., 1986. Molecular Connectivity in Structure-Activity Analysis, Research Studies Press, New York.
6. Hall, L. H., Kier, L. B., and Mohney, B., 1991. The Electrotopological State: An Atom Index for QSAR, Quant. Struct.-Act. Relat. 10: 43-51.