Toxicity of Methyl- and Halogen-Substituted Alcohols in Tissue Culture Relative to Structure–Activity Models and Acute Toxicity in Mice

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Abstract □ The relationship of *in vitro* and *in vivo* toxicity of a series of methyl- and halogen-substituted alcohols was examined with respect to their octanol–water partition coefficients (P), charge (Q), and steric (E_s) parameters. A high correlation ($r = 0.98$) was found between tissue culture toxicity (ID_{50}, the concentration required to produce 50% inhibition of growth) and hemolytic activity (H_{50}, the concentration required to produce 50% hemolysis in saline, 37°). The product of intrinsic toxicity (T_i, the slope of the dose–response curve in tissue culture) and $1/P$ for aliphatic alcohols was found to have a uniform relationship to acute *in vivo* toxicity (LD_{50}, the single intraperitoneal dose required to kill 50% of the mice in 7 days). The T_i/P to LD_{50} ratio showed a fourfold variation as compared to a 164-fold variation for the T_i/LD_{50} ratio. This relationship supported the conclusions that T_i from tissue culture was a valid, time-independent estimate of the toxicant–receptor interaction and that $1/P$ was directly related to the effective aqueous concentration of the toxicants *in vivo*. The T_i/P to LD_{50} ratio was higher for the halogenated than for the aliphatic alcohols, which suggested a difference in mechanism of the toxicant–receptor interaction. Hansch analysis showed both Q and E_s parameters to be important to the differential intrinsic toxicities of the two groups. Both Free–Wilson and Hansch–Fujita analyses supported the conclusion that the tissue culture system conforms closely to the Higuchi–Davis equilibrium model. The usefulness of the tissue culture assay in conjunction with P for the analysis and prediction of *in vivo* toxicity was demonstrated.

Keyphrases □ Alcohols, methyl and halogen substituted—*in vitro–in vivo* toxicity, octanol–water partition coefficients, tissue culture toxicity concentration–hemolytic activity correlated, toxicant–receptor interaction estimates, structure–activity relationships discussed using models □ Tissue culture assay—predictive value *in vivo*, tissue culture toxicity concentration–hemolytic activity correlated, toxicant–receptor interaction estimates, structure–activity relationships of methyl- and halogen-substituted alcohols □ Structure–activity relationships, methyl- and halogen-substituted alcohols—discussed in terms of models, tissue culture assay, toxicant–receptor interaction estimates □ Partition coefficients, methyl- and halogen-substituted alcohols—octanol–water system, correlated with biological response, tissue culture assay studies □ Lipophilicity, alcohols, methyl and halogen substituted—relationship to *in vivo* aqueous toxicant concentration, tissue culture assay

Models of structure–toxicity relationships directed toward the rationalization of *in vivo* toxicity or other pharmacological responses are necessarily simplistic in approach and are limited in applicability by the constraints of simplicity and by the availability of data consistent with those constraints. A significant limitation of current models based on correlation of physical parameters of toxicant molecules with gross biological response *in vivo* is the simultaneous dependency of response on secondary time-dependent mechanisms (*e.g.*, transport, excretion, and biotransformation) and the primary time-independent toxicant–receptor interaction, both of which are affected by the physicochemical parameters of the toxicant. Meaningful interpretation of the correlation of any given physicochemical parameter with biological response depends upon independent knowledge of the relative importance of the parameter to these two basic processes.

The linear free energy model of Hansch and Fujita (1) and Fujita *et al.* (2), the thermodynamic equilibrium model of Higuchi and Davis (3), and the purely mathematical model of Free and Wilson (4) confound time-dependent and time-independent processes, and their predictive value is limited by the validity of necessary assumptions with respect to time-dependent processes, particularly in prediction of *in vivo* response where lipophilicity of the toxicant significantly influences the effective aqueous concentration through partitioning of the toxicant to hydrophobic compartments. An independent estimate of the primary structure-dependent, time-independent toxicant–receptor interaction would, therefore, be useful in the analysis and prediction of gross biological response. The objective of the present investigation was to assess the relevance of tissue culture assay of biological activity as a means of obtaining time-independent estimates of intrinsic toxicity (T_i, the slope of the dose–response curve)[1] and its predictive value with respect to gross biological response. A series of methyl- and halogen-substituted alcohols was examined, and comparative analyses were carried out with the Free–Wilson and Hansch–Fujita models.

TISSUE CULTURE ASSAY SYSTEM

The tissue culture system conforms closely to the constraints of the equilibrium (time-independent) model of structure–activity proposed by Higuchi and Davis (3). Their conditions and fundamental assumptions with respect to the equilibrium model are discussed here with respect to the tissue culture system:

1. "A biological test system can be represented by t number of accessible compartments, w, 1, 2, 3 . . . $t + r$, where compartment w is the aqueous phase; 1, 2, 3, *etc.*, are tissue, lipoidal, protein, *etc.*, phases; and r is the receptor [specific or nonspecific]." In the mouse fibroblast monolayer tissue culture system, the predominant compartment is w, containing a quantitatively defined toxicant concentration. The r compartment is extremely small compared to

[1] "Intrinsic toxicity" is related to intrinsic activity as discussed by Ariens (5).

w, and the lipoidal, protein, *etc.*, compartments (components of the culture medium) are small and uniform relative to w. The tissue compartment (the fibroblast monolayer), also very small, is initially uniform in volume with respect to w. A maximum fivefold change in the tissue compartment occurs during the test, a very small percentage change relative to w.

2. "Thermodynamic equilibrium or quasiequilibrium is reached in all accessible phases, and the thermodynamic activity of the drug in the rth compartment is the same as that in the aqueous, first, second, *etc.*, all with reference to a common standard state. If a drug is added to the aqueous compartment, it will be distributed to all the other available compartments according to Nernst's distribution law." The tissue culture system maximizes the opportunity for such conditions to prevail during the test. Higuchi and Davis (3) concluded that the same relationships would apply to systems in which the toxicant activities are governed by rates of transport across membrane barriers, since transport rate is directly influenced by equilibrium concentration on the surface of the barrier.

3. "For a series of drugs of closely related structures, biological activity is proportional to the fraction of the active sites occupied. If the fractions occupied are made the same, then equal biological response will be elicited." The linear dose–response relationships found in the tissue culture system and the hyperbolic relationship between ID_{50}, the dose required to produce 50% inhibition of growth, and T_i are consistent with this constraint.

4. "Essentially all of the administered drug will be distributed to the various accessible body compartments, and only an insignificant amount will actually be attached to the receptor site." If the previous assumptions are valid for the tissue culture system, it is reasonable to assume that this condition also prevails.

Potentially significant factors (time dependent) such as specific (enzymatic) or nonspecific reactions that produce tangible changes in activity or concentration of the toxicant are neglected in the equilibrium model and are uncontrolled in the tissue culture system. The tissue culture system does, however, conform to a high degree to the constraints of a time-independent, quasiequilibrium test system.

These considerations apply equally well to hemolysis as a time-independent test system for the estimation of toxicity. The real time (1 hr.) of the hemolysis assay is less than 2% of the real time (72 hr.) of the tissue culture assay, and a comparison of the results with the two assays should indicate any significant differences in time dependency. If equilibrium conditions prevail in the assay systems and secondary partitioning is negligible, time dependencies of the systems would be limited to those associated directly with the kinetics of the toxicant–receptor interaction. Therefore, if the experimental assay time is invariant, the quantitative response of the system to the toxicant should provide a time-independent estimate of the intrinsic activity.

EXPERIMENTAL

Tissue Culture Assay—A method similar to that of Rosenbluth *et al.* (6, 7) was employed to evaluate the growth inhibitory response of mouse fibroblast L-cells, NCTC clone 929 strain L (Earle) (8), to the alcohol series. The medium used for maintenance of stock cultures was modified Basal Medium Eagle[2] (BME) (9) supplemented with 5% newborn calf serum, 1% L-glutamine, and 50 mcg./ml. of streptomycin sulfate. Stock cultures were subcultured every 72 hr., and cells for inoculum were harvested using 0.05% trypsin in phosphate-buffered saline. The cells for inoculum, propagated as monolayers in 900-ml. (32-oz.) prescription bottles, after trypsin treatment, were suspended in BME containing 10% serum and 2% L-glutamine by repeated forceful jetting of a 20-ml. aliquot of the BME by syringe against the monolayer. The suspended cells were then transferred to a 250-ml. conical flask containing 80 ml. of the same BME preparation and a Teflon magnetic stirring bar. A gentle rate of stirring was used to keep the cells in suspension, and cell density (cells/ml.) was estimated by hemocytometer counts. Cell density was adjusted to 2×10^6 cells/ml. with the BME preparation.

Toxicants were dissolved and diluted with BME, which contained no serum or L-glutamine, immediately prior to use. A 1-ml. aliquot of the toxicant–BME solution was added to each growth tube followed by the addition of 1 ml. of cell suspension (2.0×10^5 cells/tube). Controls (72 hr.) received the identical treatment except for the absence of toxicant. Toxicant concentrations were employed to provide growth inhibition between 10 and 90%. Four to eight toxicant concentrations were used with fivefold replication. Zero-time and 72-hr. controls were run with 10-fold replication. Zero-time controls were harvested and washed by centrifugation, $540 \times g$ for 15 min., using phosphate-buffered saline (Dulbecco solution) (10), stored at 2–4° and assayed at 72 hr.

Both treated and 72-hr. control cultures were incubated at a 10° angle in a humidified 5% CO_2–air atmosphere at 37°. The protein content of each tube was determined after decantation of the medium and washing the monolayers with phosphate-buffered saline three times. The colorimetric method of Oyama and Eagle (11) was employed for the protein determination. Percent growth was calculated as follows:

$$\% \text{ growth} = \frac{(A - B)}{(A_0 - B)} \times 100 \qquad \text{(Eq. 1)}$$

where A is the mean absorbance for cultures receiving a specific dose of toxicant, A_0 is the mean absorbance for the untreated 72-hr. control cultures, and B is the mean absorbance of the initial zero-time controls. The ID_{50} and T_i were obtained from a least-squares analysis of the dose–response data (absorbance values from the protein determinations). The adjusted ID_{50} (Adj ID_{50}) values were obtained by substituting 100% growth (for the Y intercept) into the linear equation resulting from the least-squares fit of the dose–response data.

***In Vivo* Toxicity**[3]—Male albino mice[4], ICR strain, were used to determine the LD_{50} of each compound, *i.e.*, the dose required to kill 50% of the test animals within 7 days after a single intraperitoneal injection. The experimental procedure and method of calculation were described elsewhere (12).

Partition Coefficients—Partition coefficients (Table I) were determined by the method of Hansch and Muir (13), employing equal octanol–water volumes and analyzing the concentration of the solute in the water phase or octanol phase, depending on the equilibrium concentrations in the two phases. Partitioning was carried out at four concentrations of the solute and analyzed with the system developed by Bluestein and Posmanter (14) for aqueous solutions of alcohols using a chromatograph[5].

Charge Distribution—Charge parameters (Table I) were calculated by the method of Del Re (15) for each compound in the series, employing a computer[6]. The computer program and various parameters needed for the calculations were taken from the work of Bass (16).

The charge values reported (*i.e.*, Q_0, the net charge on the hydroxyl oxygen) are in terms of electrons and were not converted to electrostatic units.

Taft's E_s Steric Parameter—The E_s values (Table I) were taken from the work of Taft (17) and were based on hydrolysis of aliphatic esters as the model reaction or the hydrolysis of *ortho*-substituted benzoic acid esters in aromatic systems. The methyl group had an assigned value of 0.0.

Free–Wilson Analysis—The method of Free and Wilson (4) was employed to analyze the structure–activity relationships in the alcohol series with three independent sets of biological response data: ID_{50}, H_{50}, and LD_{50} (Table II). One derived set of data, Adj ID_{50}, was also analyzed, the negative logarithm of the biological response being used in all analyses. The Free–Wilson substituent model for the alcohols is given in Table III, along with the substituent matrix and symmetry equations employed in the analyses.

Hansch Analysis—Utilizing the approach of Hansch and Fujita (1) and Fujita *et al.* (2), the generalized equation given in Table IV was employed in the analysis of biological response data for the alcohol series. The series was analyzed in subsets as indicated in Table IV, and the substituent model employed is given in Table V. The regression analyses were carried out with a computer[6], em-

[2] Magnesium added as magnesium chloride, 177 mg./l.

[3] Data on the *in vivo* toxicity of the alcohols employed were obtained from W. H. Lawrence, Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, Tenn.

[4] Harlan Industries, Cumberland, Ind.

[5] Beckman GC45.

[6] IBM 1620[II].

Table I—Parameters Used in Hansch Analysis of Biological Response Data

Compound[a]	Parameters[b]						
	P	Q_c	Q_0	$Q(R_1)$	$Q(R_2)$	$E_s(R_1)$	$E_s(R_2)$
1. Ethanol	0.50	0.0355	−0.4582	0.0026	0.0157	1.24	0.00
2. 1-Propanol	2.00	0.0617	−0.6411	0.0045	0.0364	1.24	−0.07
3. 2-Propanol	1.13	0.0825	−0.4615	0.0162	0.0162	0.00	0.00
4. 2-Methyl-1-propanol	5.71	0.0331	−0.4586	0.0026	0.0378	1.24	−0.47
5. 2,2-Dimethyl-1-propanol	20.78	0.0324	−0.4558	0.0026	0.0547	1.24	−0.93
6. 2-Butanol	4.07[c]	0.0812	−0.4616	0.0162	0.0248	0.00	−0.07
7. 3-Methyl-2-butanol	19.12	0.0802	−0.4618	0.0162	0.0381	0.00	−0.47
8. 3,3-Dimethyl-2-butanol	30.08	0.0794	−0.4619	0.0162	0.0549	0.00	−1.54
9. 2-Chloroethanol	1.06	0.0638	−0.4533	0.0030	0.0393	1.24	−0.24
10. 2,2-Dichloroethanol	2.34	0.0889	−0.4488	0.0034	0.0543	1.24	−1.54
11. 2,2,2-Trichloroethanol	22.30	0.1111	−0.4449	0.0038	0.0608	1.24	−2.06
12. 2-Bromoethanol	1.68	0.0586	−0.4542	0.0030	0.0400	1.24	−0.27
13. 2-Fluoroethanol	0.12	0.0522	−0.4553	0.0029	0.0688	1.24	−0.24
14. 1,1,1-Trifluoro-2-propanol	5.07	0.1227	−0.4555	0.0153	0.3836	0.00	−1.16

[a] 1-Bromo-2-propanol showed significant spontaneous chemical degradation at the time of determination of P and was eliminated from analyses involving P. Biological response data were obtained on the newly prepared compound (Table II). [b] P = octanol–water partition coefficient. The following symbols are based on the structural model (Table V): Q, charge parameters are in terms of electrons; E_s, Taft's steric parameter, see text; Q_c = charge on hydroxyl carbon; Q_0 = charge on hydroxyl oxygen; R_1 and R_2 = substituent for which the indicated parameter was calculated. [c] From *Reference 27*.

Table II—Biological Response Data for Alcohol Series

Compound	ID_{50}, M	CI[a]	Adj ID_{50}, M	T_i, %/mole	Intercept, % Growth	H_{50}[b], M	LD_{50}[c], mole/kg.
1. Ethanol	0.15540	0.00674	0.1641	−304.6	97.3	2.160	0.01208
2. 1-Propanol	0.05581	0.00197	0.0525	−950.9	103.1	0.698	0.00363
3. 2-Propanol	0.09843	0.00297	0.0983	−508.3	100.0	1.178	0.00608
4. 2-Methyl-1-propanol	0.02025	0.00129	0.0211	−2366.9	97.9	0.270	0.004208
5. 2,2-Dimethyl-1-propanol	0.01078	0.00022	0.0104	−4770.7	101.5	0.128	0.00505
6. 2-Butanol[d]	0.04300	0.00215	0.0360	−1130.0	110.0	——	0.008345
7. 3-Methyl-2-butanol	0.01439	0.00026	0.0072	−6857.2	148.7	0.168	0.00519
8. 3,3-Dimethyl-2-butanol	0.00329	0.00008	0.00255	−19566.5	114.4	0.073	0.00477
9. 2-Chloroethanol	0.03193	0.00115	0.0303	−1649.8	102.7	0.593	0.00168
10. 2,2-Dichloroethanol	0.00345	0.00015	0.0054	−9116.5	81.5	——	0.000746
11. 2,2,2-Trichloroethanol	0.00084	0.00003	0.000716	−69789.8	109.1	0.0072	0.000536
12. 2-Bromoethanol	0.00253	0.00003	0.00121	−41174.6	154.5	0.048	0.0009416
13. 2-Fluoroethanol	0.02522	0.00073	0.0250	−1997.3	100.4	——	0.000321
14. 1,1,1-Trifluoro-2-propanol	0.01492	0.00065	0.0167	−2986.1	94.6	——	0.0032877
15. 1-Bromo-2-propanol	0.00223	0.00004	0.002169	−23051.6	101.6	0.086	0.000319

[a] 95% confidence interval of $ID_{50} = ID_{50} \pm CI$. [b] Molar concentration effecting 50% hemolysis of rabbit erythrocytes; isotonic saline, 1 hr. at 37°. [c] Obtained from W. L. Lawrence, Materials Science Toxicology Laboratories (unpublished data, see text). [d] Graphical approximation of tissue culture parameters.

ploying a program based on IBM Program 06.0.148[7], "Single and Multiple Linear Regression Analyses."

RESULTS AND DISCUSSION

Biological Response Data—The primary biological response data are given in Table II. An illustrative set of dose–response curves obtained by tissue culture assay is given in Fig. 1, along with the indicated standard deviation of the primary data points. The standard deviation of the experimental data was of the order of ±5% for the 15 compounds investigated. The 95% confidence limits of the calculated ID_{50} values were also of the order of ±5%. The internal consistency of ID_{50} and T_i (hyperbolic relationship, Fig. 2) is an indication of the precision and accuracy of the tissue culture assays. It was concluded that differential evaporative loss of alcohols from the culture medium was not a significant factor in the tissue culture assay. The high correlation (r = 0.98) between the 1-hr. hemolysis assays (H_{50}) and the 72-hr. tissue culture assay (ID_{50}) was consistent with that conclusion. The precision of the H_{50} data was slightly greater than the ID_{50} data and may partly account for the higher explained variance (EV) with H_{50} data in the Hansch analysis. The higher lipophilicity of the erythrocyte membrane probably plays a significant role with respect to the higher absolute magnitude of toxicant required to produce an equivalent response in the hemolysis assay. The LD_{50} data had 95% confidence limits of ±10% or less.

[7] Provided by the Department of Molecular and Quantum Biology, University of Tennessee Medical Units, Memphis, Tenn.

Free–Wilson Analysis—A high degree of explained variance was obtained with ID_{50} data (92%) and H_{50} data (94%) with the Free–Wilson mathematical model (Table VI). Since the absolute times of the H_{50} assay and ID_{50} assay were significantly different, the consistency of results and the high degree of explained variance with the two sets strongly support the time independence of those assay systems. The poor fit of the Free–Wilson model with LD_{50} data was not surprising and indicated significant secondary interactions, shown by Hansch analysis to be associated with P, charge parameters, and steric parameters. This is consistent with the conclusion of Singer and Purcell (18) as to when the Free–Wilson model would be expected to fail.

The substituent group activities, as calculated from the Free–Wilson analysis of the ID_{50} data, are given in Table VII in order of decreasing activity. As expected from receptor theories, the parent portion of the molecule contributes the major portion of the biological activity. Calculated ID_{50} values are compared to observed values in Fig. 2. The only compound falling off the regression significantly was 2-fluoroethanol; this finding is consistent with its known conversion to a more active intermediate. The fact that the other compounds were very close to the calculated regression suggested that metabolic conversion did not play a major role in the toxicity of those compounds. The decrease in explained variance with Adj ID_{50} data is not consistent with the hypothesis of significant secondary partitioning of toxicant in the tissue culture system and supports the conclusion of time independence of the system. The theoretical ID_{50} for 2-butanol was calculated from substituent activities obtained in the analysis of ID_{50} values for the other members of the series prior to experimental determination of the ID_{50}. The values differed by 18%, indicating the predic-

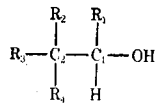


Table III—Substituent Model Employed in Free–Wilson Analysis

Compound	Substituents at C_1[a]		Substituents at C_2[b]				
	H	CH_3	H	CH_3	Cl	Br	F
1. Ethanol	1		3				
2. 1-Propanol	1		2	1			
3. 2-Propanol		1	3				
4. 2-Methyl-1-propanol	1		1	2			
5. 2,2-Dimethyl-1-propanol	1			3			
6. 2-Butanol (excluded)[c]							
7. 3-Methyl-2-butanol		1	1	2			
8. 3,3-Dimethyl-2-butanol		1		3			
9. 2-Chloroethanol	1		2		1		
10. 2,2-Dichloroethanol	1		1		2		
11. 2,2,2-Trichloroethanol	1				3		
12. 2-Bromoethanol	1		2			1	
13. 2-Fluoroethanol	1		2				1
14. 1,1,1-Trifluoro-2-propanol		1					3
15. 1-Bromo-2-propanol		1	2			1	

[a] Symmetry equation: position C_1, $-9H + 5CH_3 = 0$. C_1 = substituent position one. [b] Symmetry equation: position C_2, $-19H + 11CH_3 + 6Cl + 2Br + 4F = 0$. C_2 = substituent position two. [c] The Free–Wilson analysis was used to calculate the activity of this compound.

tive value of the Free–Wilson model for low molecular weight alcohols *in vitro*.

Hansch Analysis—The structural model employed for Hansch analysis is given in Table V. The following physical parameters (Table I) were used in the analysis. The octanol–water partition coefficient, P, was employed as a basic estimate of the relative lipophilicity of the compounds. The charge on the oxygen atom (Q_0), the charge on the hydroxyl carbon (Q_c), the charge on the R_1 substituent [$Q(R_1)$], and the R_1 steric parameter [$E_s(R_1)$] were associated with the hydroxyl end of the molecules. The R_2 steric parameter [$E_s(R_2)$] and the charge parameter [$Q(R_2)$] were associated with the net properties about the C_2 carbon which was included in the R_2 substituent. The results (EV) of selected single and multiple linear correlations of those parameters with biological response are given in Table IV.

The linear dependence of the ID_{50} of aliphatic alcohols on P (Fig. 3) is consistent with the conclusions that the tissue culture system is in quasiequilibrium and that the correlations with physical parameters obtained using the Hansch model relate primarily to the toxicant interactions with the mouse fibroblast L-cell receptor system. The consistency of the correlative results obtained with the ID_{50} and H_{50} lends further support to these conclusions. The regression of $-\log ID_{50}$ and $-\log H_{50}$ on $\log P$, considering all compounds, gave regression coefficients of 0.513 and 0.805 and intercepts of 0.554 and 0.134, respectively, indicating a greater sensitivity (19) of the hemolysis assay than the tissue culture assay to changes in P. The high degree of correlation of ID_{50} and H_{50} data and the general consistency of explained variance between the two sets with respect to physical parameters are consistent with the current concept of nonspecific membrane activity of alcohols (20). A linear dependency of $-\log$ biological response on $\log P$ should be expected when a one-step partitioning process is involved, as with external receptor sites of a cell membrane or in enzymatic studies (21). The linear dependence found for the aliphatic alcohols is consistent with that found by Hansch and Anderson (22). The generally lower explained variance obtained with LD_{50} data and the differential results *in vivo* and *in vitro* with respect to correlation with specific physical parameters are indicative of qualitative differences in the toxicant–receptor interaction *in vivo* and *in vitro*. Although qualitative differences were apparent in the Hansch analysis, the uniformity of the T_i/P to LD_{50} ratios within subhomologies of the alcohol series suggested that the differences were small (Tables VIII and IX).

Considering all *in vitro* data, P gave the highest overall single-parameter explained variance but gave no significant explained variance with *in vivo* data. The $Q(R_2)$ was the least quantitatively reliable parameter, but it was the only single parameter that gave a statistically significant explained variance with *in vitro* and *in vivo* data. However, the explained variance for halogenated alcohols *in vitro* decreased significantly as compared to the explained variance with P.

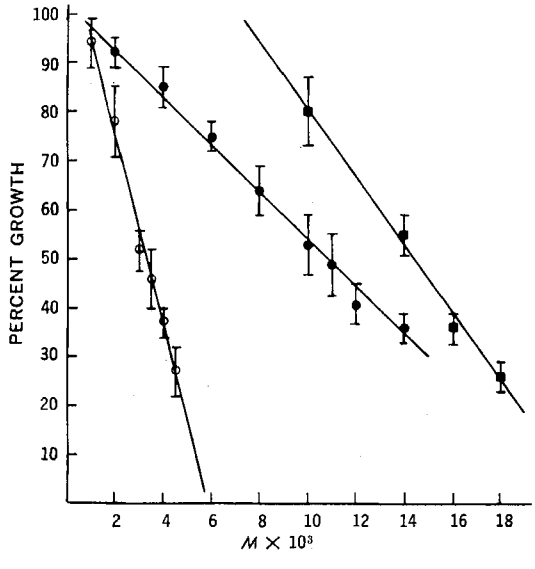


Figure 1—*Illustrative dose–response curve obtained for three alcohols by tissue culture assay. Key:* ●, *2,2-dimethyl-1-propanol;* ■, *3-methyl-2-butanol; and* ○, *3,3-dimethyl-2-butanol.*

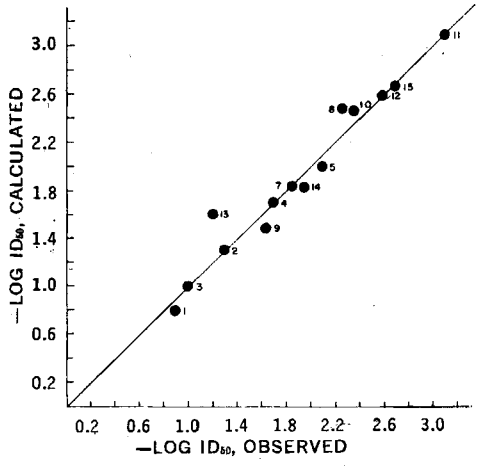


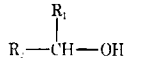
Figure 2—*Correlation of calculated $-\log ID_{50}$ (Free–Wilson analysis) and $-\log$ of experimentally observed ID_{50} (Table II).*

Table IV—Explained Variance of Biological Response Using Selected Equations of the General Form: $-\log BR = k(\log P)^2 = k_1 \log P + k_2 Q_x + k_3(E_s)_x + k_4$

	ID_{50}	Adj ID_{50}	H_{50}	LD_{50}	ID_{50}	Adj ID_{50}	H_{50}	LD_{50}	ID_{50}	Adj ID_{50}	H_{50}	LD_{50}	ID_{50}	Adj ID_{50}	H_{50}	LD_{50}
	Variable: P				Variables: P^2,P				Variables: $Q_c,E_s(R_2)$				Variables: $P^2,P,E_s(R_1)$			
All alcohols	0.25	0.27	0.46	−0.06[a]	0.23	0.26	0.40	−0.15[a]	0.63	0.46	0.66	−0.15[a]	0.30	0.29	0.46	−0.22[a]
Secondary	0.80	0.95	—	−0.22[a]	0.71[a]	0.93	—	−0.81[a]	0.84	0.66[a]	—	0.63	—	—	—	—
All aliphatic	0.91	0.94	0.98	0.16[a]	0.91	0.95	0.98	0.26[a]	0.85	0.79	0.70	−0.17[a]	0.89	0.94	0.97	0.19[a]
Secondary	0.86	0.96	0.96	0.07[a]	0.85	0.98	0.93	0.77[a]	0.998	0.96[a]	0.999	−0.38[a]	—	—	—	—
Primary	0.98	0.99	0.99	0.07[a]	0.98	0.99	0.99	0.78[a]	0.76[a]	0.78[a]	0.80[a]	0.05[a]	—	—	—	—
All halogenated	0.38[a]	0.27[a]	—	−0.20[a]	0.23[a]	0.08[a]	—	0.25[a]	0.33[a]	−0.02[a]	—	−0.11[a]	0.36[a]	0.16[a]	—	−0.05[a]
Primary	0.61	0.50[a]	—	−0.32[a]	0.44[a]	0.26	—	0.05[a]	0.17[a]	−0.25[a]	—	−0.41[a]	−0.11[a]	—	—	—
	Variable: $Q(R_2)$				Variables: P,Q_c				Variables: $Q_0,E_s(R_2)$				Variables: $P^2,P,Q(R_2)$			
All alcohols	−0.07[a]	−0.08[a]	0.68	−0.05[a]	0.24	0.25	0.48	−0.16[a]	0.63	0.47	0.65	−0.16[a]	0.16[a]	0.19[a]	0.61	−0.25[a]
Secondary	−0.27[a]	−0.33[a]	—	0.55	0.81	0.94	—	0.52[a]	0.84	0.66[a]	—	0.62	0.81[a]	0.98	—	0.85
All aliphatic	0.82	0.74	0.81	0.33	0.90	0.94	0.98	−0.01[a]	0.84	0.73	0.68	0.34[a]	0.91	0.93	0.98	0.47[a]
Secondary	0.999	0.96	0.95	0.22	0.86[a]	0.93[a]	0.99	−0.62[a]	0.98	0.997	0.94[a]	−0.36[a]	—	—	—	—
Primary	0.84	0.88	0.88	0.25	0.96[a]	0.97	0.98	0.45[a]	0.77[a]	0.80[a]	0.81[a]	0.14[a]	—	—	—	—
All halogenated	−0.16[a]	−0.13[a]	—	−0.16[a]	0.41[a]	0.46[a]	—	−0.47[a]	0.35[a]	0.05[a]	—	−0.36[a]	0.34[a]	0.16[a]	—	−0.06[a]
Primary	−0.33[a]	−0.33[a]	—	0.67	0.42[a]	0.33[a]	—	0.80	0.17[a]	−0.25[a]	—	−0.42[a]	0.05[a]	−0.47[a]	—	0.03[a]
	Variable: Q_c				Variables: P,Q_0				Variables: $Q(R_1),Q(R_2)$				Variables: $P^2,P,E_s(R_2)$			
All alcohols	0.09[a]	0.06[a]	0.13[a]	−0.08[a]	0.26	0.27	0.43	−0.16[a]	−0.13[a]	−0.16[a]	0.65	−0.07[a]	0.59	0.44	0.73	−0.19[a]
Secondary	−0.33[a]	−0.33[a]	—	0.43[a]	0.81	0.94	—	0.50[a]	0.999	0.95	—	0.59[a]	0.99	0.9999	—	−0.36[a]
All aliphatic	−0.15[a]	−0.12[a]	−0.15[a]	−0.16[a]	0.90	0.93	0.98	0.53	0.92	0.91	0.91	0.20	0.99	0.95	0.998	0.22[a]
Secondary	0.92	0.96	0.99	−0.01[a]	0.92[a]	0.99	0.95[a]	0.39[a]	0.998	—	—	—	—	—	—	—
Primary	−0.34[a]	−0.38[a]	−0.37[a]	−0.21[a]	0.96[a]	0.97	0.98	0.48[a]	0.87[a]	0.89[a]	0.90[a]	0.28[a]	—	—	—	—
All halogenated	−0.08[a]	−0.18[a]	—	−0.25[a]	0.44[a]	0.18[a]	—	0.34[a]	−0.14[a]	−0.11[a]	—	−0.07[a]	−0.13[a]	−0.36[a]	—	0.61[a]
Primary	0.43	0.17[a]	—	−0.19[a]	0.42[a]	0.32	—	0.80	0.33[a]	0.01[a]	—	0.52[a]	−0.07[a]	−0.41[a]	—	0.41[a]
	Variable: Q_0				Variables: $P,Q(R_1)$				Variables: $P,Q_c,Q(R_2)$				Variables: P^2,P,Q_c			
All alcohols	0.02[a]	0.01[a]	−0.02[a]	−0.08[a]	0.32	0.30	0.51	−0.12[a]	0.18[a]	0.19[a]	0.71	−0.25[a]	0.24[a]	0.25[a]	0.40	−0.26[a]
Secondary	−0.33[a]	−0.33[a]	—	0.42[a]	0.81	0.94	—	0.51[a]	0.995	0.98	—	0.12[a]	0.79[a]	0.97[a]	—	0.89[a]
All aliphatic	−0.11[a]	−0.11[a]	−0.11[a]	0.10[a]	0.90	0.93	0.98	0.05[a]	0.91	0.93	0.98	0.15[a]	0.89	0.94	0.97	−0.08[a]
Secondary	0.94	0.997	0.97	0.26[a]	0.71[a]	—	—	—	—	—	—	—	—	—	—	—
Primary	−0.42[a]	−0.44[a]	−0.44[a]	−0.09[a]	0.96	0.97	0.98	0.48[a]	—	—	—	—	—	—	—	—
All halogenated	0.50	0.29	—	−0.02[a]	0.55[a]	0.43[a]	—	−0.48[a]	0.29[a]	0.21[a]	—	0.72[a]	0.28[a]	0.36[a]	—	−0.06[a]
Primary	0.43[a]	0.18[a]	—	−0.18[a]	0.44[a]	0.26[a]	—	0.78[a]	0.29[a]	0.43[a]	—	0.68[a]	−0.10[a]	−0.13[a]	—	0.84[a]
	Variable: $E_s(R_2)$				Variables: $P,Q(R_2)$				Variables: $E_s(R_1),E_s(R_2)$				Variables: $P,Q_c,E_s(R_2)$			
All alcohols	0.65	0.51	0.68	−0.07[a]	0.18[a]	0.20[a]	0.65	−0.14[a]	0.65	0.47	0.65	−0.12[a]	0.59	0.43	0.62	−0.17[a]
Secondary	0.78	0.55	—	0.40[a]	0.82	0.94	—	0.53[a]	—	—	—	—	0.995	0.996	—	0.27[a]
All aliphatic	0.86	0.78	0.74	0.02[a]	0.92	0.93	0.98	0.22[a]	0.85	0.78	0.70	−0.14[a]	0.97	0.97	0.98	−0.24[a]
Secondary	0.87	0.73	0.70[a]	0.29[a]	0.998	0.995	0.98	−0.49[a]	—	—	—	—	—	—	—	—
Primary	0.81	0.79	0.81	−0.32[a]	0.97	0.97	0.98	−0.28[a]	—	—	—	—	—	—	—	—
All halogenated	0.34[a]	0.09[a]	—	−0.11[a]	0.52[a]	0.42[a]	—	−0.52[a]	0.36[a]	0.03[a]	—	−0.22[a]	0.56[a]	0.37[a]	—	0.36[a]
Primary	0.45[a]	−0.15[a]	—	−0.11[a]	0.50[a]	0.26[a]	—	0.51[a]	0.17[a]	—	—	—	0.84[a]	0.62[a]	—	0.83[a]
	Variables: $Q_0,E_s(R_1)$				Variables: $P,E_s(R_2)$				Variables: $Q(R_2),E_s(R_2)$				Variables: $P^2,P,Q(R_1)$			
All alcohols	−0.03[a]	−0.06[a]	−0.11[a]	−0.12[a]	0.63	0.48	0.65	−0.08[a]	0.65	0.50	0.71	0.14	0.29	0.29	0.45	−0.22[a]
Secondary	—	—	—	—	0.99	0.994	—	0.29[a]	0.82	0.62[a]	—	0.63[a]	0.80[a]	0.97[a]	—	0.88[a]
All aliphatic	−0.29[a]	−0.24[a]	−0.29[a]	−0.07[a]	0.98	0.96	0.99	0.00[a]	0.89	0.78	0.80	0.39[a]	0.89	0.94	0.97	0.16[a]
Secondary	—	—	—	—	0.995	0.996	0.98	−0.41[a]	0.998	0.99	0.99	−0.42[a]	—	—	—	—
Primary	—	—	—	—	0.96[a]	0.98	0.98	0.99	0.83[a]	0.86[a]	0.87[a]	0.48[a]	—	—	—	—
All halogenated	0.34[a]	0.05[a]	—	−0.31[a]	0.23[a]	0.03[a]	—	0.55[a]	0.38[a]	0.06[a]	—	−0.29[a]	0.36[a]	0.17[a]	—	−0.06[a]
Primary	0.14[a]	—	—	—	0.46[a]	0.26[a]	—	0.48[a]	0.28[a]	−0.11[a]	—	0.53[a]	−0.11[a]	−0.32[a]	—	0.60[a]

[a] Less than 90% statistical confidence by F test.

Table V—Substituent Model Employed for Hansch Analyses



Compound	R_1	R_2
1. Ethanol	H	CH_3
2. 1-Propanol	H	C_2H_5
3. 2-Propanol	CH_3	CH_3
4. 2-Methyl-1-propanol	H	iso-C_3H_7
5. 2,2-Dimethyl-1-propanol	H	*tert*-C_4H_9
6. 2-Butanol	CH_3	C_2H_5
7. 3-Methyl-2-butanol	CH_3	iso-C_3H_7
8. 3,3-Dimethyl-2-butanol	CH_3	*tert*-C_4H_9
9. 2-Chloroethanol	H	$ClCH_2$
10. 2,2-Dichloroethanol	H	Cl_2CH
11. 2,2,2-Trichloroethanol	H	Cl_3C
12. 2-Bromoethanol	H	$BrCH_2$
13. 2-Fluoroethanol	H	FCH_2
14. 1,1,1-Trifluoro-2-propanol	CH_3	F_3C

The importance of charge on the hydroxyl end of the molecule can be seen in the correlations with Q_0 and P,Q_0, the latter giving the highest explained variance "overall" *in vitro* and *in vivo*. The two-parameter fit with $P,Q(R_2)$ gave a slightly increased overall explained variance *in vitro*, particularly for the halogenated alcohols as compared to $Q(R_2)$ alone. The importance of substituents at C_2 is apparent in the correlation with $P,E_s(R_2)$, which gave significant explained variance for all compounds *in vitro*, an excellent fit *in vivo* for primary aliphatic alcohols (99.5%), and a substantial explained variance (although of low statistical confidence) for the halogenated alcohols. The significance of the methyl group in the C_1-position was reflected in the $P,E_s(R_2)$ statistics for the primary and secondary aliphatic alcohols and suggested that the steric aspects of the receptor site favor the hydrogen substituent at C_1. The possible significance of steric parameters was suggested by Hansch *et al.* (23). It is significant that $P,E_s(R_2)$ gave a substantially higher explained variance *in vivo* for the aliphatic primary alcohols than the P^2,P correlation. This suggests that toxicant–receptor interactions are of primary importance *in vivo* and the explained variance is of the same high order of magnitude as for the *in vitro* correlations where there are nonstatistical reasons to believe that the biological response is essentially dependent on the toxicant–receptor interaction. Although P^2,P correlations for both primary and secondary aliphatic alcohols *in vivo* gave 78 and 77% explained variance, respectively, the partial regression coefficients for P^2 were −0.495 and 0.342, respectively, which raises a question as to the physical significance of the P^2 term (24). The difference in sign of the coefficients and the highly significant difference in explained variance between the primary and secondary aliphatic alcohol with $P,E_s(R_2)$ *in vivo* indicated a significant qualitative difference between the *in vitro* and *in vivo* systems. However, the magnitude of this difference cannot be greater than that responsible for the fourfold variation observed in the T_i/P to LD_{50} ratios for those compounds (discussed below). The correlation with $E_s(R_2)$ alone gave a slightly lower overall explained variance *in vitro* than $P,E_s(R_2)$ but gave a significantly lower explained variance with *in vivo* data, indicating a qualitative difference *in vitro* and *in vivo*.

The high, statistically significant explained variance (94–99.7%) *in vitro* for the secondary aliphatic alcohols was associated with relatively high positive values of Q_c as compared to from −42 to −44% explained variance for the primary aliphatic alcohols associated with significantly lower Q_c values. Halogenation of the primary aliphatic alcohols induces a higher positive charge on the hydroxyl carbon, and the explained variance for that set increased to 43% (statistically significant). Although the absolute values of Q_0 do not vary as much as Q_c, the same statistical relationship exists for that parameter and, as pointed out previously, contributes significantly to the P,Q_0 correlation. The correlations with $Q_c,E_s(R_2)$ and $Q_0,E_s(R_2)$ gave a generally lower explained variance than $P,E_s(R_2)$. This was not surprising because P depends upon the overall physical properties of the molecules and has greater predictive value than single parameters associated with specific or localized molecular properties. Low order correlations with specific parameters, although not expected to give high explained variances, serve to isolate those factors of importance to net biological response.

For any significant improvement in overall explained variance for the halogenated alcohols, a three-parameter fit using P, Q_c, and $E_s(R_2)$ was required. This suggested the importance of both charge and steric interactions; however, the statistical confidence of the correlations was less than 90%. The generally lower explained variance obtained for the halogen-substituted alcohols can be rationalized in terms of the heterogeneity of that set of compounds. Except for mono-, di-, and trichloroethanol, there is no systematic homology of substitution; each of the other members of the set represents single members of different subhomologies with different mechanisms of action, as indicated by the T_i/P to LD_{50} ratios.

The Adj ID_{50} values were calculated on the assumption that the variation in the percent growth intercept reflected a variation in the effective concentration of toxicant as a result of partitioning of the toxicant to nonaqueous compartments (the cell monolayer and components of the medium). Although the Free–Wilson results tended to support the assumption of nonsignificant partitioning in the cell culture system, the results of the Hansch analysis on the Adj ID_{50} data indicated that there may have been a small amount of toxicant loss due to partitioning for the secondary (high partitioning) aliphatic alcohols. In general, there is a slight decrease in the Adj ID_{50} explained variance or no change at all as compared with the ID_{50} explained variance. This is probably a result of the random statistical error in the intercept being greater than the real, but small, partitioning effect *in vitro*.

Linear equations relating biological response and physical parameters, which give a high degree of explained variance, have value for the prediction of biological response of untested con-

Table VI—Statistical Results of Free–Wilson Analyses

Statistical Parameters	Biological Response: ID_{50}	Adj ID_{50}	H_{50}[a]	LD_{50}
r^{2}[b]	0.95	0.93	0.97	0.54
Explained variance (*EV*), %	92	88	94	25
Significance of *F* ratio, %	99.9	99.9	99.9	90

[a] Biological response for 11 compounds. 2-Butanol, 2,2-dichloroethanol, 2-fluoroethanol, 1,1,1-fluoro-2-propanol, and 1-bromo-2-propanol were not included. [b] The square of the coefficient of multiple correlation, *r*.

Table VII—Substituent Group Contribution to ($-\log ID_{50}$)

Substituent	Activity ($-\log ID_{50}$) Dependent Variables, Methyl[a] and Bromine[b]
Substituent position C_1	
Methyl	0.085323
Hydrogen	−0.047402
Substituent position C_2	
Bromine	1.330441
Chlorine	0.409717
Methyl	0.089450
Fluorine	−0.014229
Hydrogen	−0.318222
Overall average, μ	1.911543

[a] At substituent position C_1. [b] At substituent position C_2.

Table VIII—Relationship of T_i, P, and LD_{50}: Aliphatic Alcohols

Compound	$[T_i/LD_{50}] \times 10^{-4}$	$[(T_i/P)/LD_{50}] \times 10^{-4}$
1. Ethanol	2.5	5.0
2. 1-Propanol	26.2	13.1
3. 2-Propanol	8.4	7.4
4. 2-Methyl-1-propanol	56.3	9.9
5. 2,2-Dimethyl-1-propanol	94.5	4.6
6. 2-Butanol	13.5	3.3
7. 3-Methyl-2-butanol	132.2	6.9
8. 3,3-Dimethyl-2-butanol	410.2	13.6

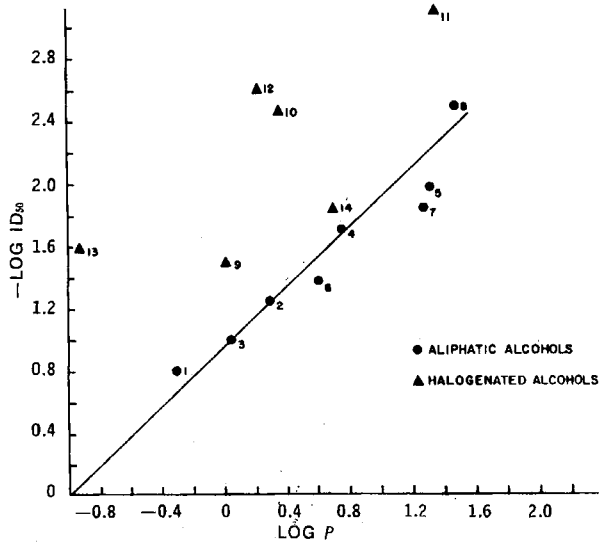


Figure 3—*Correlation of −log ID_{50} and log* P. (*For key, see Table II.*)

geners. Selected equations giving the highest explained variance for the alcohol series investigated are given in Table X.

Time Independence of T_i and ID_{50}—The essentially perfect hyperbolic relationship between T_i and ID_{50} (*i.e.*, linear relationship between ID_{50} and $1/T_i$) (Fig. 4) gives strong support for the conclusion that equilibrium conditions prevail in the tissue culture system. That T_i bears this direct relationship to cellular toxicity, the ID_{50}, is consistent with the conclusion that it provides an estimate of the time-independent interaction or affinity of the toxicant with the receptor system of the cell. The overlap of the intrinsic toxicities of the methyl-substituted and halogen-substituted members of the series, divergent in chemical and physical attributes, within the hyperbolic distribution is further indication that T_i is an estimate of the toxicant–receptor interaction rather than secondary factors such as diffusion. The linearity of the dose–response relationship in the tissue culture was consistent with the assumptions of equilibrium and that the assay conditions were such that a first-order relationship prevailed in the toxicant–receptor interaction. The findings of Fink and Bender (25) regarding the kinetics and mechanism of inhibition of papain-catalyzed

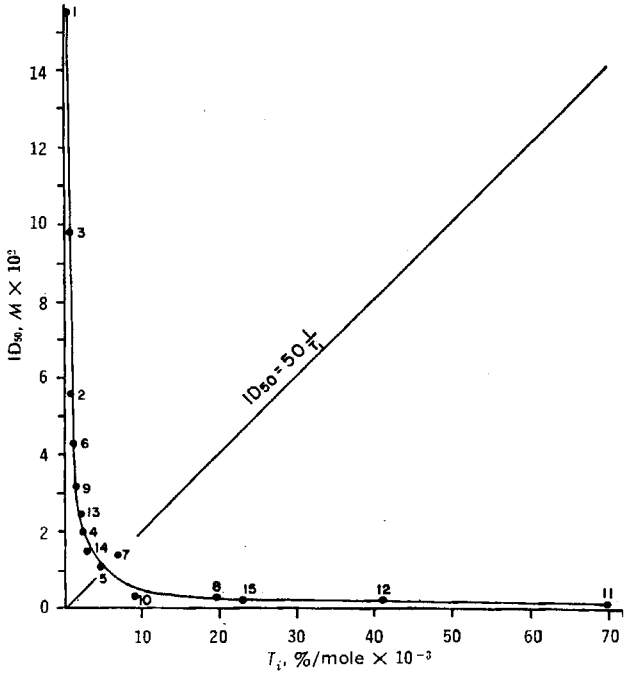


Figure 4—*Correlation of ID_{50} and* T_i. (*For key, see Table II.*)

Table IX—Relationship of T_i, P, and LD_{50}: Halogenated Alcohols

Compound	$[T_i/LD_{50}] \times 10^{-4}$	$[(T_i/P)/LD_{50}] \times 10^{-4}$
9. 2-Chloroethanol	98.2	92.7
10. 2,2-Dichloroethanol	1222.1	522.2
11. 2,2,2-Trichloroethanol	13020.4	583.9
12. 2-Bromoethanol	437.3	260.3
13. 2-Fluoroethanol	622.2	5185.0
14. 1,1,1-Trifluoro-2-propanol	90.8	17.9

hydrolyses by a series of straight-chain, primary alcohols is consistent with the observed tissue culture response (T_i and ID_{50}) obtained with the alcohol series in the present investigation. The shift in the kinetics observed in the enzyme system in the presence of an increasing molecular weight of the alcohols coincided with the hyperbolic break in ID_{50} values obtained in tissue culture in this investigation, which gives further indirect support to the conclusion that the tissue culture system reflects a toxicant–receptor interaction.

Relationship of Tissue Culture Intrinsic Toxicity (T_i) and Acute Toxicity (LD_{50}) in the Mouse—The hyperbolic relationship of T_i and ID_{50} (Fig. 4) relates to the degree of inhibition or saturation of the receptor system and not to the mechanism of the inactivation. If the receptor system of the mouse fibroblast cell in tissue culture is an adequate model for the receptor system in the mouse and if quasiequilibrium conditions attain in the 7-day LD_{50} determination, the product of T_i and the equilibrium concentration of the toxicant in the aqueous phase (proportional to $1/P$) should have a direct and constant relationship:

$$\frac{T_i/P}{LD_{50}} = \text{constant } (C) \qquad \text{(Eq. 2)}$$

where:

T_i = time-independent intrinsic toxicity of the toxicant
P = time-independent octanol–water partition coefficient of the toxicant (assuming the octanol–water system to be an adequate model of the partitioning system *in vivo*)
LD_{50} = single intraperitoneal dose required to kill 50% of the test animals in 7 days (which assumes percent kill to be directly proportional to the number of receptor sites inactivated)

If T_i is a valid time-independent estimate of intrinsic toxicity, experimental variations in C reflect the time-dependent reactions or processes *in vivo* that affect equilibrium concentration of toxicant, intrinsic toxicity of the toxicant (through biotransformation), and constancy of the receptor system.

The ratio of T_i to LD_{50} varied 164-fold and 143-fold for the aliphatic and halogenated alcohols, respectively (Tables VIII and IX). The inclusion of P in the T_i/P to LD_{50} ratio reduced the variation to fourfold for the aliphatic alcohols, indicating that the T_i/P relationship largely accounted for the *in vivo* toxicity of that series. Involved in the fourfold variation was the statistical accuracy of the parameters, the fact that T_i and LD_{50} were expected to vary inversely, and the fact that the ratio was the result of dividing large numbers by small numbers. It was, therefore, not possible to ascribe the observed variations to time dependencies, but the magnitude of time dependencies that exist must fall within the observed variation in the ratio.

The ratios obtained with the halogenated alcohols were higher than for the aliphatic alcohols and had a significantly higher range of variation overall. This was consistent with the greater heterogeneity of structural substituents within that set. It also suggested a qualitative difference in the toxicant–receptor interaction as compared to the aliphatic alcohols or a qualitative difference in the receptor systems affected by the two sets. The qualitative differences between the sets observed in the Hansch analysis, particularly with respect to charge parameters, and the consistency of the hyperbolic relationship of T_i and ID_{50} suggested that the primary receptor system was the same for the two sets. It was significant that the magnitude of the deviations of the halogenated set from the linear regression of $-\log ID_{50}$ on $\log P$ for aliphatic alcohols (Fig. 3) generally followed the shift in magnitude of the T_i/P to LD_{50} ratio, which was consistent with the conclusion that the variation

Table X—Linear Regression of Biological Activity on Physical Parameters: Selected Equations

	EV, %
All compounds	
$-\log ID_{50} = -0.787E_s(R_2) + 0.310$	65
$-\log$ Adj $ID_{50} = -0.751E_s(R_2) + 0.380$	51
$-\log H_{50} = 0.805 \log P + 0.134$	46
$= -0.875E_s(R_2) + 0.166$	68
All secondary alcohols	
$-\log ID_{50} = 0.904 \log P - 0.042$	80
$= 0.549 \log P - 0.432E_s(R_2) - 0.028$	99
$-\log$ Adj $ID_{50} = 1.06 \log P - 0.084$	95
$= 0.860 \log P - 0.242E_s(R_2) - 0.076$	99
$-\log LD_{50} = 0.342 \log P^2 - 0.409 \log P + 9.25Q_c - 1.53$	89
$= 0.350 \log P^2 - 0.421 \log P + 62.56Q_0 + 28.10$	88
All aliphatic alcohols	
$-\log ID_{50} = 0.820 \log P - 0.011$	91
$= 0.501 \log P - 0.445E_s(R_2) + 0.01$	98
$-\log$ Adj $ID_{50} = 0.917 \log P - 0.018$	94
$-\log H_{50} = 0.772 \log P - 0.093$	98
$-\log LD_{50} = 0.178 \log P - 1.69Q_0 - 1.70$	53
All primary aliphatic alcohols	
$-\log ID_{50} = 0.735 \log P + 0.049$	98
$-\log$ Adj $ID_{50} = 0.751 \log P + 0.042$	99
$-\log H_{50} = 0.770 \log P - 0.078$	99
$-\log LD_{50} = -0.495 \log P^2 + 0.712 \log P - 0.803$	78
$= 1.04 \log P + 1.40E_s(R_2) - 0.768$	99
All secondary aliphatic alcohols	
$-\log ID_{50} = 0.932 \log P - 0.122$	86
$= 0.497 \log P - 0.484E_s(R_2) - 0.003$	99
$-\log$ Adj $ID_{50} = 1.07 \log P - 0.124$	96
$= 0.809 \log P - 0.294E_s(R_2) - 0.052$	99
$-\log H_{50} = 0.778 \log P - 0.121$	96
$= -388.22Q_c + 31.93$	99
$-\log LD_{50} = 0.342 \log P^2 - 0.427 \log P - 0.769$	77
All halogenated alcohols	
$-\log ID_{50} = 0.592 \log P + 1.00$	38
$= 115.07Q_0 + 53.19$	50
$-\log LD_{50} = 0.292 \log P^2 - 1.40 \log P + 43.86Q_c - 5.50Q(R_2) - 2.93$	89
All primary halogenated alcohols	
$-\log ID_{50} = 0.704 \log P + 1.10$	61
$= 1.57 \log P - 701.65Q_0 - 2.85E_s(R_2) - 318.22$	90
$= 1.52 \log P - 114.26Q_c - 2.61E_s(R_2) + 7.22$	84
$-\log$ Adj $ID_{50} = 0.721 \log P + 1.14$	50
$= 1.97 \log P - 136.18Q_c - 2.79E_s(R_2) + 8.65$	62
$= 2.04 \log P - 836.34Q_0 - 3.08E_s(R_2) - 379.25$	62
$-\log LD_{50} = -1.38 \log P + 48.27Q_c - 3.39$	80
$= -1.36 \log P + 269.82Q_0 + 121.99$	80

in the ratio reflects a variation in the toxicant–receptor interaction rather than secondary time dependencies. The variation in ratios of T_i to LD_{50} for the halogenated set was of the same order of magnitude as for the aliphatic set and, excluding the fluorine-substituted compounds, the sixfold variation of the T_i/P to LD_{50} ratios was of the same order of magnitude as for the aliphatic set, indicating again the significance of T_i and P in the rationalization of *in vivo* response. The significantly higher ratio for 2-fluoroethanol is consistent with its known conversion to an intrinsically more toxic intermediate (26). The ratio for 1,1,1-trifluoro-2-propanol was only slightly higher than for the aliphatic series. Structurally, it resembles more closely its homolog, 2-propanol, in the aliphatic series than the other halogenated alcohols resemble their respective homologs because of the distinctly larger size of the chlorine and bromine substituents. The significance of substituent size can be seen in the mono-, di-, and trichloroethanol ratios and in the fact that the bromoethanol ratio falls between mono- and dichloroethanol. This is consistent with the significant contribution of $E_s(R_2)$ in the Hansch analysis.

The T_i/P to LD_{50} ratio reflects qualitative similarities or differences in mechanism and has value in the prediction of *in vivo* toxicity when the magnitude of the ratio is established for a well-defined series of congeners, as in the aliphatic series of alcohols. In this study, the ID_{50} of 2-butanol was predicted prior to the experimental determination of the LD_{50}. By using the theoretically calculated ID_{50} from the Free-Wilson analysis, T_i was estimated from the hyperbolic relationship of ID_{50} and T_i (Fig. 4) and was used in conjunction with P and the average value of the T_i/P to LD_{50} ratio for secondary aliphatic alcohols to predict the LD_{50} of 2-butanol. The predicted value was a factor of 2 lower than the experimentally determined LD_{50} but was within the 95% confidence limits of the comparison and well within the fourfold variation of the aliphatic set.

When LD_{50} is plotted against T_i, the basic hyperbolic relationship is observed (Fig. 5), with the high partitioning secondary alcohols and primary alcohols showing significantly lower toxicity than expected, resulting from active partitioning into the hydrophobic compartments of the animal. The position of 1,1,1-trifluoro-2-propanol falls between the secondary aliphatic alcohols and the lower segment of the hyperbolic curve made up primarily of the halogenated compounds. This relationship was reflected in the T_i/P to LD_{50} ratio already discussed. 1,1,1-Trifluoro-2-propanol is a single member of a subhomology, and mono- and difluoro-2-propanol would be expected to fall on a curve between 2-propanol and 1,1,1-trifluoro-2-propanol; significant deviation from this relationship would indicate an alteration in mechanism by metabolic conversion or other specific secondary reactions affecting T_i, as found in the case of 2-fluoroethanol.

The "hyperbolic" relationship of LD_{50} and T_i is not as precise as that found in the ID_{50} and T_i relationship. The LD_{50} values through which the curve is drawn are high with respect to a true hyperbolic relationship. The magnitude of the deviations for the high partitioning halogenated alcohols was less than those found in the aliphatic set, which is consistent with the suggested qualitative

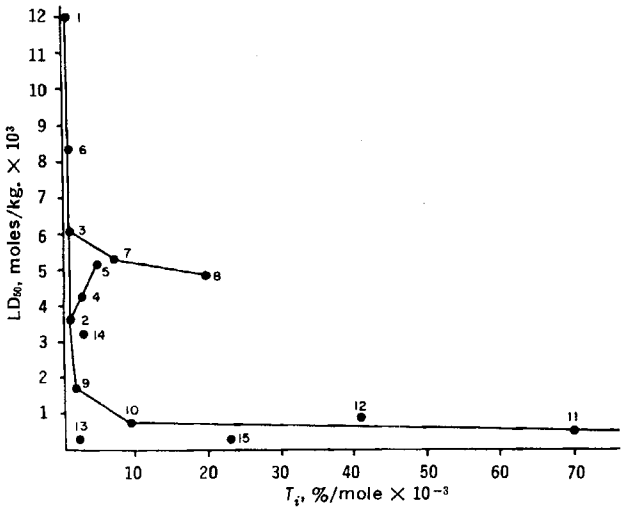


Figure 5—*Correlation of* LD_{50} *and* T_i. (*For key, see Table II.*)

difference in mechanism for the halogenated compounds. The general hyperbolic relationship of LD_{50} and T_i and the fact that the octanol–water partition coefficient serves as a basis for the rationalization of *in vivo* response with respect to T_i indicate a significant relationship of *in vitro* T_i to *in vivo* response. They also indicate the pertinence of the Higuchi equilibrium model for the rationalization of *in vivo* response where estimates of effective aqueous concentration of toxicant are available. The significant correlation of *in vitro* and *in vivo* toxicity through the T_i/P to LD_{50} relationship strongly supports the assumption that P is the predominant factor in determining the time course of toxicant effect *in vivo* and that the LD_{50} based on mortality data at 7 days provides adequate time for expression of dose-related primary toxicity. This seems reasonable in view of the relatively rapid assimilation and excretion of low molecular weight alcohols. Variations in the time dependencies of these processes no doubt contribute to the four- to sixfold variation in the T_i/P to LD_{50} ratios.

CONCLUSIONS

The results of this investigation support the conclusion that intrinsic cellular toxicities obtained by tissue culture assay of methyl- and halogen-substituted alcohols are directly related to the intrinsic *in vivo* toxicities of those compounds. This conclusion rests on the relative uniformity of the T_i/P to LD_{50} ratio within subhomologies of the series and supports the conclusion that $1/P$ is directly related to the effective aqueous concentration of toxicant *in vivo*. This relationship also supports the conclusion that the tissue culture system is in quasiequilibrium and provides valid estimates of T_i. Major differences in the T_i/P to LD_{50} ratios between the aliphatic and halogenated alcohols are attributed primarily to differences in toxicant–receptor interactions rather than secondary time-dependent processes. Qualitative differences in *in vivo* and *in vitro* toxicity are detectable by Hansch analysis, but the quantitative contributions of those differences cannot exceed that responsible for the relatively small variation in the T_i/P to LD_{50} ratios observed within the subhomologies.

Hansch analysis of the biological response data leads to the conclusion that P and $E_s(R_2)$ are the most significant physical parameters associated with biological response of primary aliphatic alcohols and that $E_s(R_1)$ is of primary importance to the biological response of the secondary aliphatic alcohols. A higher positive charge at C_1, associated with the methyl substituent at C_1 and with halogenation at C_2, is a significant factor in the toxicant–receptor interaction, and a qualitative difference exists between *in vitro* and *in vivo* response relative to that parameter. Charge on the hydroxyl oxygen, Q_o, is related to that phenomenon and is of primary importance in the toxicant–receptor interaction.

The excellent fit of *in vitro* data to the Free–Wilson model and the results of Hansch analysis support the conclusion that the tissue culture system is a quasiequilibrium, time-independent system.

The usefulness of tissue culture intrinsic toxicity, T_i, and the octanol–water partition coefficient, P, for the analysis and prediction of *in vivo* response is demonstrated.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 11, 1972, from the *Materials Science Toxicology Laboratories, College of Pharmacy and College of Dentistry, University of Tennessee Medical Units, Memphis, TN 38103*

Accepted for publication July 25, 1972.

Presented to the Society of Toxicology, Williamsburg, Va., March 1972.

Supported by National Institute of Dental Research Grant DE02944 and by U. S. Public Health Service Graduate Training Grant GM-02052.

The contribution by Dr. W. H. Lawrence of LD_{50} values used in this investigation is gratefully acknowledged. The technical assistance of Dr. David Chambers, Dr. Munir Pesh-Imam, and Mrs. Jane Parker is acknowledged.

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