QSAR Analysis and Data Extrapolation among Mammals in a Series of Aliphatic Alcohols

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Concepts of QSAR analysis and biological similarity models are combined for use in extrapolation of LD₅₀ values after IP application of a series of aliphatic alcohols (C₃–C₉) to mouse, hamster, rat, and guinea pig and rabbit. It has been found that although close correlation exists between LD₅₀ values after IP and IV applications for mouse and rat, the QSARs obtained with LD₅₀ after IV application are not suitable for a prediction of LD₅₀ values after IP application for rabbit. Different transformation or distribution processes in mouse, rat, and rabbit after the two types of applications might be the reason.

The LD₅₀ values (expressed in mmole/m² of body surface) seem to be independent of mammalian species used (at least within the mouse, rat, hamster, and probably guinea pig series). This fact makes it possible to predict reasonable values of LD₅₀ after IP application for rabbit. Expression of toxicity in mmole/m² of body surface may be useful in toxicological studies.

The model of quantitative structure–activity–species relationships (QSASR) for the system of alcohols and animals chosen is proposed:

\[
\log BA_i = k_j + l_j \log X_i \\
\log BA_j = a_j + b_j \log Z_j
\]

where \(i\) denotes an alcohol, \(j\) an animal, \(BA\) being LD₅₀ (mmole/m²) after IP application, \(X\) molecular connectivity \(X\) and \(Z\) body surface: body weight ratio. The model is based on the assumption that \(b_j\) is independent of chemical structure (being zero or close to zero), \(a_j\) is a function of molecular connectivity \(X\), \(k_j\) and \(l_j\) being independent of animal species. These assumptions resulted from the statistical analysis of QSARs and allometric equations obtained under various conditions.

Introduction

An enormous effort has been devoted to solving the problem how to extrapolate data obtained on one animal to another animal or even to man. The results of a number of tests on biological models and experimental animals are extrapolated to man mostly taking into account relative differences in body weight or in body surface on the supposition that man reacts similarly to the model.

For a description of differences in physiological functions among various species, empirical allometric equations were suggested based on a biological similarity model (1–4). The toxic responses of several toxicants studied as a quantitative function of body weight within one animal species demonstrate that weight may be used for the extrapolation of data on toxic tests from one size animal to another size (5). It was reported (6, 7) that the relationship between response and dose can be best expressed when the independent variable is plotted as a total amount given each animal. Other investigators advocated that the dose should be corrected by a two-thirds power of body weight ("surface area factor"). This factor proved to be useful, e.g., for a prediction of lethal toxicity of antineoplastic agents for different size animals not only within, but also among several mammalian species (8). The following equation is in accordance with the proposition (9) to relate dosage to an exponent of body weight that need not be necessarily two-thirds:

\[
Y = a + b \log M/W^h
\]

where \(Y\) is survival time after dosage \(M\) of sodium arsenite ingested by silkworm larvae of different size and development, \(W\) represents some measure of body size, \(h\) an exponent that can be defined as a ratio of regression

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slopes of the relationships between the response and the quantities of dose and body weight (9).

Equation (2) represents the customarily used formula:

\[ \log C = \log a + b \log W \]  

(2)

showing a linear relationship between \( \log \) of body weight \((W)\) and \( \text{LD}_{50}\) or \( \text{LC}_{50} \) \((C)\). Antilog form of Equation (2)

\[ C = aW^b \]  

(3)

resembles the allometric formulae of Huxley (3). It supports the idea that the allometric formulae can be used not only to describe a quantitative relationship between body weight and rates of physiological processes or anatomical structures, but also pharmacological or toxicological activities (10–12). The usefulness of the allometric Equation (2) as a mode of depicting \( \text{LD}_{50}\) or \( \text{LC}_{50} \) values has been shown over a wide range of various animals (5). Nevertheless, the extrapolations are often quite empirical on the basis of analogies and experiences with similar compounds.

The idea of expressing the relationship between a xenobiotic toxic activity and body weight, as a parameter of an animal species, might be comparable with that originating with the hypothesis leading to the formulation of QSAR, i.e., to the formulation of quantitative approaches to biological activity–chemical structure relationships (13–17). The ideas on which the QSAR analysis are based suggest that both approaches, i.e., QSAR analysis and the analysis using allometric equations, may be combined for the extrapolation of data on biological tests among compounds and animal species (11,12). It means that a quantitatively expressible relationship between xenobiotic toxic activity and "structure" of both the xenobiotic and the animal species can be expected:

\[ \log \text{BA}_{ij} = k_{ij} + l_i \log X_i \]  

(4)

\[ \log \text{BA}_{ij} = a_j + b_i \log Z_j \]  

(5)

or another form of the equations, where \( \text{BA}_{ij} \) denotes an activity of a xenobiotic \( i \) on a biological object \( j \), \( X_i \) a structural characteristic \( X \) of the xenobiotic \( i \) (e.g., \( n \)-octanol/water or oil/air partition coefficient, quantum chemical indices, molecular connectivity, etc.), \( Z_j \) a parameter \( Z \) of the biological object \( j \) (e.g., body weight, body surface, a metabolic activity, distribution volumes, etc.) (11,12).

This approach may be useful in the extrapolation of data among biological species; the predicted values of xenobiotics from one animal to another are checked by the whole system of formulae connecting a series of xenobiotics with a series of animals. It might reveal outliers caused by disparate metabolism or transport of the xenobiotic or caused by different experimental conditions.

The aim of this paper is to demonstrate the power of the proposed quantitative model: toxicity–chemical structure–biological object for the extrapolation of data among biological objects. For this purpose, \( \text{LD}_{50}\) values of a series of aliphatic alcohols (C1–C5) obtained with mice, rats, hamsters, guinea pigs, and rabbits have been determined after IP and IV applications and QSARs as well as interspecies correlations have been derived. Values of \( \text{LD}_{50}\) for rabbit after IP application are estimated and their validity is discussed.

**Materials and Methods**

**Experimental Animals**

The animals were taken from a controlled breeding animal farm at the Research Institute for Pharmacy and Biochemistry or from a farm in Velaz: male mice of the strain H, 20–24 g, male rats of the strain Wistar, 200–240 g, male Syrian hamsters, 190–250 g, guinea pigs of both sexes and of various origins, 350–500 g, Chinchilla rabbits of both sexes, 2500–3500 g. The animals had free access to water during the experiment and were fed with a common diet.

**Alcohols Applied**

Methanol, ethanol, \( n \)-propanol, isopropanol, \( n \)-butanol, isobutanol, sec-butanol and \( n \)-pentanol, analytical grade, checked by gas chromatography to have less than 1% impurities, were dosed in aqueous solutions. Higher alcohols, heptanols and octanols, which are poorly soluble in water, were not used for the study because of less reproducible doses as their aqueous suspensions.

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**Table 1A. Constants used for conversion of \( \text{LD}_{50}\) values to molar doses.**

|          | MeOH | EtOH | n-ProOH | i-ProOH | n-BuOH | i-BuOH | s-BuOH | n-PenOH |
|----------|------|------|---------|---------|--------|--------|--------|---------|
| Molecular weight | 32.04 | 46.07 | 60.10 | 60.10 | 74.13 | 74.13 | 74.13 | 88.16 |
| Density, g/mL   | 0.7914 | 0.7989 | 0.8036 | 0.7864 | 0.8102 | 0.8020 | 0.8063 | 0.8146 |

**Table 1B. Constants used for conversion from body weight units to body surface units.**

|          | Mouse | Rat | Hamster | Guinea pig | Rabbit |
|----------|-------|-----|---------|-------------|--------|
| \( K, \text{ m}^2/\text{kg}^a \) | 0.2250 | 0.1790 | 0.1591 | 0.1306 | 0.0876 |
| Body weight, g^b | 22 | 200 | 250 | 400 | 3500 |

^a Data from Spector (21).

^b An average body weight of the species for which the constant \( K \) is chosen.
**Determination of 50% Lethal Doses (LD<sub>50</sub>)**

LD<sub>50</sub> values were determined from the mortality observed 5 days after an application in one laboratory (Research Institute for Pharmacy and Biochemistry) unless described otherwise. The aqueous solutions of alcohols were used for both IV and IP application. The doses were adjusted by changing the sample volume used, the concentration of the dosing solution remaining constant. Several concentrations (the lowest and the highest ones differed approximately two-fold) of the same alcohol were used to find if there was a dependence of LD<sub>50</sub> values on the concentration applied.

The LD<sub>50</sub> values and their 95% confidence intervals were calculated by an approximate graphic probit method (18–20). In some cases the number of animals used for the determination was too small for using the graphic probit method. Then, an approximate interval LD<sub>0</sub>–LD<sub>100</sub> was found and LD<sub>50</sub> taken as an arithmetical mean of the LD<sub>0</sub>–LD<sub>100</sub> interval, which was considered as the 95% confidence interval (guinea pigs, rabbits).

The LD<sub>50</sub> values determined in mL of 100% alcohol/kg of body weight were converted to mmole/kg or to mmole/m<sup>3</sup> of body surface using the constants given in Table 1.

Pooling of data from individual experiments was carried out using the method of weighted means in cases where no significant differences among them were found by using the χ<sup>2</sup>-test (e.g., no dependence of LD<sub>50</sub> on concentrations applied, etc.)

**Statistical Evaluation**

An agreement or a difference between the experimental characteristics (LD<sub>50</sub>) was tested by χ<sup>2</sup>-test estimating the variances of log LD<sub>50</sub> from their 95% confidence interval, among regression equations by χ<sup>2</sup>-test using the estimated covariance matrices of regression coefficients.

The regression equations between experimental characteristics were computed by the weighted least-squares method considering the fact that both variables are due to an error of known quantity (variance of the characteristics). The goodness of fit was tested by χ<sup>2</sup>-test. When the deviations from a predicted line were significant, the variances of estimated regression coefficients were adjusted by the heterogeneity factor. The significance of regression coefficients was tested by the t-test using the adjusted variance.

**Molecular Connectivity Indices**

Molecular connectivities of the zero order, 0<subΧ</sub>, and of the first order, 1<subΧ</sub>, were calculated by a common way proposed by Randić (22) and modified by Kier and Hall (23) for QSAR analysis:

\[ 0_{\chi} = \sum \frac{1}{\sqrt{\delta_r}}, \quad 1_{\chi} = \sum \frac{1}{r_{ss} \sqrt{\delta_s}} \]

where δ<sub>r</sub> and δ<sub>s</sub> are valence atomic connectivities of all atoms r forming an alcohol molecule or of all neighboring atoms r and s in the molecule (i.e., overall bonds in the molecule), their values being 1 for C(H3), 2 for C(H2), 3 for C(H) and 5 for O(H) (Table 2).

**Table 2.** Molecular connectivities used in the QSAR analysis of LD<sub>50</sub> after intraperitoneal application of a series of aliphatic alcohols.

| Alcohol   | 0<subΧ</sub> | 1<subΧ</sub> |
|-----------|--------------|--------------|
| MeOH      | 1.447        | 0.447        |
| EtoH      | 2.154        | 1.023        |
| n-ProOH   | 2.961        | 1.923        |
| i-ProOH   | 3.024        | 1.412        |
| n-BuOH    | 3.568        | 2.023        |
| i-BuOH    | 3.731        | 1.878        |
| s-BuOH    | 3.731        | 1.950        |
| n-PenOH   | 4.275        | 2.923        |

**Table 3. Primary experimental values of LD<sub>50</sub> of a series of aliphatic alcohols after IP application and the estimated values.**

| Alcohol | Mouse | Rat | Hamster | Guinea pig<sup>b</sup> | Rabbit |
|---------|-------|-----|---------|---------------------|--------|
| MeOH Exptl | 336(299,373) | 237(222,252) | 327(235,304) | 111(74.1,148) | — |
| EtoH Exptl | 137(120,154) | 81.4(72.7,89.9) | 129(120,137) | 137(103,171) | — |
| n-ProOH Exptl | 61.5(46.1,76.9) | 37.4(34.8,40.1) | 38.9(36.2,41.6) | 20.1(13.4,26.7) | 8.57(6.43,10.2) |
| i-ProOH Exptl | 81.6(65.4,96.8) | 47.1(33.9,60.2) | 57.7(47.2,68.2) | 74.1(34.3,160) | 20.9(18.4,23.8) |
| n-BuOH Exptl | 74.5(63.9,89.3) | 45.3(42.0,49.2) | 57.3(45.4,72.4) | 42.6(19.8,92.1) | 11.1(9.51,12.9) |
| s-BuOH Exptl | 24.3(20.4,29.0) | 18.9(15.8,22.8)* | 18.9(15.0,23.7) | 16.2(5.50,35.0) | 4.36(3.80,5.00) |
| n-PenOH Exptl | 20.6(16.8,25.4) | 16.1(9.27,27.8) | 16.4(13.0,20.8) | 14.4(6.66,30.9) | 3.74(2.44,5.72) |

<sup>*A weighted mean and its 95% confidence interval; the experimental values are the first line of each pair, the estimated ones, the second line. Values of LD<sub>50</sub> obtained from a comparatively small group of animals (4–6 animals for a dose).<sup>

<sup>Estimated value from LD<sub>100</sub> (24): LD<sub>50</sub> = 1.2 LD<sub>100</sub>.<sup>

<sup>Statistically significant difference between the experimental and the estimated values at p < 0.05.
Table 4. Experimental and the estimated values of LD_{50} of a series of aliphatic alcohols after IP application.

| Alcohol | Mouse | Rat | Hamster | Guinea pig | \(\chi^2\)-test | LD_{50}, mmole/m², rabbit |
|---------|-------|-----|---------|------------|-----------------|------------------------|
| MeOH    | Exptl | 1489(1397,1583) | 1499(1319,1707) | 851(568,1136) | 7.739 | 1474(1390,1598) |
|         | Estd  | 1571(1391,1871) | 1803(1426,2280) | 154(102,265)  | 5.767 | 227(208,246)    |
| EtOH    | Exptl | 609(533,684) | 512(457,566) | 723(675,771) | 1050(788,1313) | 43.581 | 660(545,799) |
|         | Estd  | 588(489,694) | 548(505,596) | 618(489,782) | 568(283,1226) | 4.982 | 557(490,634) |
| n-PrOH  | Exptl | 273(205,342) | 235(189,252) | 219(103,234) | 154(102,265) | 8.677 | 227(208,246) |
|         | Estd  | 231(194,275) | 206(184,256) | 239(184,256) | 246(114,331) | 7.366 | 228(171,272) |
| i-PrOH  | Exptl | 390(291,378) | 296(213,378) | 324(295,353) | 1376 | 332(306,361) |
|         | Estd  | 331(280,397) | 286(264,309) | 322(255,407) | 327(152,706) | 10.417 | 295(253,344) |
| n-BuOH  | Exptl | 77.8(63.1,92.4) | 89.3(68.5,110) | 66.3(48.3,78.4) | 92.1(68.6,126) | 3.692 | 78.8(69.0,90.1) |
|         | Estd  | 99.0(83.1,118) | 94.5(87.4,102) | 87.7(56.2,137) | 106(49.1,229) | 2.089 | 94.6(72.8,125) |
| i-BuOH  | Exptl | — | 61.0(26.1,149) | — | — | — | — |
|         | Estd  | 108(90.7,129) | 119(95.0,143) | 124(57.5,268) | 29.474 | 116(101,133) |
| s-BuOH  | Exptl | — | — | — | — | — | — |
|         | Estd  | 91.774(5,113) | 101(58.3,175) | 92.2(72.9,117) | 110(51.0,237) | 185(631 | 99.4(65.1,152) |
| n-PenOH | Exptl | — | 42.1(33.4,53.0) | — | — | — | — |
|         | Estd  | 49.1(41.2,58.5) | 41.3(39.4,44.2) | 39.3(28.1,56.6) | 53.5(24.8,115) | 38.966 | 42.3(35.2,50.9) |

*aA weighted mean and its 95% confidence interval. The values are calculated from the original values expressed in mmole/kg presented in Table 3.

Values of \(\chi^2\)-test of a difference among the values of LD_{50} of the four species investigated.

Estimated values; the higher values are estimated from the experimental LD_{50} values of mouse, rat, hamster, and guinea pig as a weighted mean; the lower values from the values estimated for the four species.

Estimated from value of LD_{50} (22), LD_{50} = 1.2 LD_{50} (2).

Statistically significant difference between the experimental and the estimated values at \(p < 0.05\).

Results

The primary set of experimental LD_{50} values (mmole/kg) of aliphatic alcohols C1–C5 after IP and IV applications are summarized in Tables 3 (the first line of data, IP) and 5 (the first line of data, IV). The LD_{50} values converted to body surface (mmole/m²) are presented in Tables 4 (the first line of data, IP) and 5 (the second line of data, IV). No dependence of LD_{50} values on a concentration applied has been found; therefore all observations were included in the calculation regardless of the concentration (30–50 animals for one dose in the case of mice and rats, 10–20 for hamsters). Figure 1 qualitatively demonstrates a dependence of LD_{50} values (mmole/m², IP) on length of alkyl chains in alcohols.

In the next step we have completed the matrix of LD_{50} values after IP application (where more data than after IV application have been collected) with data estimated using methods of QSAR analysis or allometric equations.

A statistically significant correlation was found between LD_{50} values (mmole/m²) obtained after IV application and those after IP application with mice and rats (Table 6) and between LD_{50} values (mmole/m², IP) and molecular connectivity index of the first order \(\chi\) of the alcohols (Table 6). No correlation was found with the zero-order molecular connectivity \(\chi\). The log LD_{50} value correlation for guinea pig was less significant because of a large 95% confidence interval due to the small number of animals used for determining LD_{50}. The LD_{50} values obtained after IP application showed interspecies correlations among the animal under study (i.e., mouse, rat, hamster, and guinea pig) being least significant in the case of guinea pig (Table 7).

Three LD_{50} estimates (mmole/m², IP) were obtained

Table 5. Experimental values of LD_{50} of a series of aliphatic alcohols after their IV application.

| Alcohol | Mouse | Rat | Rabbit |
|---------|-------|-----|--------|
| MeOH    | 147(126,171) | 66.5(61.5,71.2) | 278(185,271) |
|         | 653(560,760) | 418(367,448) | 734(697,985) |
| EtOH    | 48.0(43.9,52.6) | 39.5(34.5,43.8) | 51.5(34.3,68.5) |
|         | 213(195,234) | 248(223,275) | 137(911,1823) |
| n-PrOH  | 11.6(8.82,15.2) | 9.82(7.65,12.5) | 8.04(6.69,9.36) |
|         | 51.6(39.2,67.7) | 61.7(41.1,85.6) | 219(178,249) |
| i-PrOH  | 25.1(23.9,26.3) | 18.1(17.0,19.3) | 19.7(16.4,22.9) |
|         | 112(106,117) | 114(107,121) | 522(435,609) |
| n-BuOH  | 6.07(5.25,7.76) | 4.18(3.64,4.86) | — |
|         | 27.0(23.3,34.5) | 26.0(22.9,30.5) | — |
| i-BuOH  | 5.63(4.33,7.24) | 4.59(4.32,4.86) | — |
|         | 25.0(19.2,32.2) | 28.8(27.2,30.5) | — |
| s-BuOH  | — | 1.86(0.98,3.26) | — |
|         | — | 11.7(6.16,20.5) | — |
| n-PenOH | 3.23(3.03,3.41) | 2.22(1.85,2.67) | — |
|         | 14.4(13.5,15.2) | 14.0(11.6,16.8) | — |

*aThe weighted mean and 95% confidence interval. The first of each pair of lines is LD_{50} values expressed in mmole/kg; the second line is LD_{50} in mmole/m².

The values of LD_{50} obtained from a small group of rabbits (2–3 animals for a dose), for which only an interval LD_{50} can be determined. The values of LD_{50} are taken as the arithmetical mean.

for each alcohol and animal in the matrix using LD_{50} IV–LD_{50} IP intercorrelations, interspecies correlations (especially with LD_{50} of mouse and rat) and correlations with molecular connectivity \(\chi\). As no statistically significant difference were found among those three esti-
mates for the individual cases, they were included in one weighted average with its estimated 95% confidence interval (Table 4, the second line of data and Fig. 1). Their values given in mmole/kg of body weight are summarized in Table 3 (the second line of data).

No significant difference was found between the experimental LD₅₀ values and those estimated by the way described above with the only exception of i-BuOH for rat. Even the estimates of LD₅₀ values (mmole/m², IP) of MeOH for guinea pig were satisfactory because of a wide 95% confidence interval for the estimates.

Statistically highly significant agreement was found among experimental LD₅₀ values of individual alcohols expressed in mmole/m² units after IP application for all four animal species (Table 4, the column χ²-test). Such agreement among LD₅₀ values was not found if they were expressed in mmole/kg units (Table 3) or after IV application (Table 5). The LD₅₀ values for rat (mmole/m², IP) were, thus, estimated as weighted means of the experimental LD₅₀ values (Table 4, the first line in the column, "Rabbit") or of the estimated ones (Table 4, the second line in the column, "Rabbit") for mouse, rat, hamster, and guinea pig. In Table 3 containing the primary set of experimental LD₅₀ values, the estimates for rabbit are given as their averages.

Discussion

The results summarized in Tables 3, 4, 6, and 7 support the suggestion that QSAR analysis can be helpful in an extrapolation of toxic indices among various animal species. Several ways for extrapolation of LD₅₀ values of aliphatic alcohols after IP application have been followed: use of a similarity between regression equations describing a relation between log LD₅₀ (mmole/m²) and molecular connectivity \( \chi \) after both types of application used (IP and IV) for animals studied (mouse, rat, and hamster); use of a similarity of intercorrelations between LD₅₀ values of various species after IP and IV applications; to employ LD₅₀ after IV application using intercorrelations between LD₅₀ values obtained after IV and IP applications; to employ allometric equations, i.e., to find a relation between log LD₅₀ (IP) and a characteristic parameter of animal species.

Tables 7 and 8 show a similarity between the regression equations describing intercorrelations between LD₅₀ values for mouse and rat after IP and IV applications.

Table 6. Constants of regression equations correlating log LD₅₀ (mmole/m²) after IP application with log LD₅₀ (mmole/m²) after IV application or with the first-order molecular connectivity \( \chi \).

| \( y = bx + a \) | \( y \) | \( x \) | \( b \) | \( a \) | \( n \) | SD | \( \chi^2 \) | \( t_0 \) | Species |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| log LD₅₀ (IP)   | log LD₅₀ (IV) | 0.834 ± 0.113 | 0.840 ± 0.254 | 6 | 0.073 | 56.57 | 11.115 | Mouse |
| log LD₅₀ (IV)   | \( \chi \) | -0.768 ± 0.118 | 3.546 ± 0.135 | 5 | 0.071 | 89.35 | -10.129 | Mouse |
| log LD₅₀ (IV)   | \( \chi \) | -0.751 ± 0.032 | 3.505 ± 0.035 | 5 | 0.018 | 15.34 | -37.279 | Rat |
| log LD₅₀ (IV)   | \( \chi \) | -0.868 ± 0.152 | 3.683 ± 0.239 | 5 | 0.083 | 313.21 | -6.714 | Hamster |
| log LD₅₀ (IV)   | \( \chi \) | -0.736 ± 0.059 | 3.508 ± 0.766 | 4 | 0.278 | 237.41 | -1.964 | Guinea pig |
| log LD₅₀ (IV)   | \( \chi \) | -0.795 ± 0.057 | 3.157 ± 1.026 | 6 | 0.046 | 162.12 | -21.274 | Mouse |
| log LD₅₀ (IV)   | \( \chi \) | -0.808 ± 0.152 | 3.072 ± 0.231 | 8 | 0.140 | 157.63 | -8.119 | Rat |
| log LD₅₀ (IV)   | \( \chi \) | -1.381 ± 0.470 | 4.543 ± 0.642 | 4 | 0.184 | 255.25 | -8.377 | Rabbit |

*The constants are given ± 1.96 SE corrected for the value of \( \chi^2 \)-test. \( n \) is the number of data pairs in the correlation; SD is the standard deviation of the estimate; \( \chi^2 \) values of \( \chi^2 \)-test, \( t_0 \) values of \( t \)-test of the regression coefficient \( b \).*

**Not significant.**
Table 7. Matrix of constants of regression equations correlating log LD₅₀ (mmole/m²) values obtained after IP application for the animal species studied.*

|       | Mouse          | Rat            | Hamster        | Guinea pig     |
|-------|----------------|----------------|----------------|----------------|
| Mouse | 0.981 ± 0.131  | 0.908 ± 0.140  | 0.855 ± 0.543  |                |
|       | 0.067 ± 0.371  | 0.245 ± 0.392  | 0.385 ± 1.466  |                |
|       | 44.271 ± 5     | 48.626 ± 5    | 213.138 ± 5    |                |
|       | 0.062 ± 10.45  | 0.068 ± 9.383  | 0.215 ± 2.346  |                |
| Rat   | 1.006 ± 0.134  | 0.934 ± 0.277  | 0.753 ± 0.575  |                |
|       | -0.050 ± 0.384 | 0.097 ± 0.605  | 0.642 ± 1.546  |                |
|       | 45.402 ± 5     | 153.146 ± 5   | 255.169 ± 5    |                |
|       | 0.062 ± 10.35  | 0.117 ± 4.858  | 0.270 ± 2.049  |                |
| Hamster | 1.061 ± 0.168 | 0.995 ± 0.294  | 0.928 ± 0.458  |                |
|        | -0.215 ± 0.467 | 0.083 ± 0.8333 | 0.193 ± 1.234  |                |
|        | 57.283 ± 5     | 160.611 ± 5   | 160.248 ± 5    |                |
|        | 0.080 ± 8.566  | 0.128 ± 4.698  | 0.900 ± 0.438  | 0.237 ± 2.909  |
| Guinea pig | 0.902 ± 0.572 | 0.926 ± 0.688  | 0.900 ± 0.438  |                |
|        | 0.263 ± 1.538  | 0.218 ± 1.824  | 0.266 ± 1.178  |                |
|        | 261.061 ± 5    | 401.820 ± 5   | 180.900 ± 5    |                |
|        | 0.288 ± 2.293b | 0.323 ± 1.936b | 0.230 ± 2.991b |                |

*For each block of values the first line is the regression coefficient b ± 1.96 SE corrected for the value of x² test; the second line is the constant a ± 1.96 SE corrected for the value of x²-test of the regression equation y = bx + a; the first value on the third line is the x²-test; the second value in the third line is the number of data pairs; The first value on the fourth line is the SD of the estimate, and the second value in the fourth line is the t-test of the regression coefficient b.

bNot significant.

If one tries to apply this fact for the intercorrelations between LD₅₀ values of rabbit and mouse or of rabbit and rat (Table 8), estimates of LD₅₀ (mmole/m², IP) for rabbit were too high, e.g., as high as about 40 mL/kg of MeOH or 5 to 6 mL/kg of n-PrOH. Table 6 indicates a similarity between the regression equations describing the relationships between log LD₅₀ (mmole/m²) and molecular connectivity X₂ after IP and IV applications for mouse and rat, the constant a being higher by about 0.4 log units in the case of IP application. Applying this to the rabbit (after IV application, the last line of Table 6) leads again to unreal estimates (30–35 mL/kg for MeOH or about 2 mL/kg for n-PrOH). Thus, we have found no way to extrapolate LD₅₀ values for rabbit obtained after IV application to estimate those after IP application, although a close correlation between these two types of LD₅₀ values exists in the case of mouse and rat (Table 6, the first two lines) and undoubtedly exists even for rabbit. This might be explained by differences in transformation or distribution processes in these three species (mouse, rat, and rabbit) after IP and IV applications of an alcohol.

Another striking similarity exists among LD₅₀ values of each of the alcohols studied for all animal species chosen if they are expressed in mmole/m² units (Table 4). No significant difference can be found among the LD₅₀ values of any of the alcohols for mouse, rat, and hamster. Those in guinea pig sometimes show differences, but their wide 95% confidence interval makes them comparable with the others. By using a weighted mean as a prediction for rabbit (which virtually simulates an allometric equation), LD₅₀ values of about 2.3 mL/kg for MeOH or about 0.6 mL/kg for n-PrOH are obtained, which are much more reasonable than the predictions mentioned above.

Let us continue to define a quantitative relationship between LD₅₀ (mmole/m², IP) and a parameter of the animal species chosen [Eq. (5)]. The type of LD₅₀ values used is independent of the animal tested, but dependent on the chemical structure of the alcohol. Therefore the species parameter may be arbitrarily chosen, e.g., body weight, body surface or their ratio. We have chosen the log form of the body surface: body weight ratio (unpublished results). The regression equations

\[
\log LD₅₀ \text{ (mmole/m², IP)} = f(\log \text{body surface: body weight})
\]

have a regression coefficient of about zero and an intercept with the LD₅₀ axis that is close to the estimates given in Table 4.
Figure 2. Graphic representation of the quantitative structure-activity-species relationships for the system: log LD_{so} (mmole/m^2, IP)-aliphatic alcohols (C_1-C_5)-mammals (mouse, rat, hamster, guinea pig, rabbit). The intercept of the model plane with the xy-plane is described by the line representing a regression equation of a dependence of log LD_{so} on \chi common to mouse, rat, hamster, guinea pig (and rabbit): log LD_{so} = -0.78 \chi + 3.56, that with the yz-plane by the line parallel to the z-axis at y = 3.56.

Figure 2 schematically illustrates the situation showing a plot of log LD_{so} (mmole/m^2, IP) against both molecular connectivity \chi (parameter of the chemical structure) and body surface: body weight ratio (parameter of animal species). It is represented by a plane that intersects the LD_{so}-body surface: body weight plane in a line parallel to the body surface: body weight axis and the log LD_{so}-\chi plane in a line described by the regression equation log LD_{so} = f(\chi) (Table 6).

Using the hypothesis published earlier (11,12) (Eqs. 4 and 5), it is possible to conclude from the study of this system of alcohols, animals and LD_{so} (mmole/m^2, IP) that: the constant \(b_1\) is not dependent on chemical structure of alcohols, being close to zero; the constant \(a_1\) is a linear function of molecular connectivity \chi (close to -0.78 \chi + 3.56); the constants \(l_j\) and \(k_j\) are not dependent on the parameter used for the description of animal species, i.e., body surface: body weight ratio being \(l_j = -0.78, k_j = 3.56\).

This rather simple example points out advantages of the QSASR hypothesis suggested earlier (11,12), but a large number of difficulty obtainable experimental results necessary for a construction of the model remains, however, an unpleasant disadvantage. A determination of additional LD_{so} values is necessary to prove that the model is valid in the whole scale of the system chosen.

This study also indicates that the expression of the magnitude of toxic effects in units of mmole/m^2 might often be more helpful than that expressed in mmole/kg units.

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