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COMPUTER-ASSISTED 'PROBACENT' FORMULAS PREDICTING TOLERANCE OF MICE TO METRAZOL AND ELECTROSHOCK: USE OF APPLE COMPUTER WITH CALCLINE PROGRAM AND METHOD TO CONSTRUCT

Probacent Formulas in Biomedical Research Sung Jang Chung[1*]
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Abstract:-

Calcline computer program is applied to construct the author's 'probacent' formulas with use of Apple computer, MacBook that express the mathematical relationship among the drug dose of Metrazol, the time after administration and the percentage of mortality or among the voltage of electroshock, the duration and the percentage of occurrence of convulsion in mice. Analysis of the actual and the computer-assisted predicted mortality or percentage of occurrence of convulsion in mice has shown a remarkable agreement and a fair accuracy (p>0.995 in chi square goodness-of-fit test). The method how to construct the 'probacent' formula in biomedical research is described in detail in this study.

Keywords: *Computer program; Calcline; Probacent formula, Formula of mortality, Formula of convulsion; Drug tolerance; electroshock; Apple computer.*

1. INTRODUCTION

Selye (1936) introduced the idea of stressor to stress-producing stimuli for living bodies (e.g. cold, heat, traumatic injury, burns, radiation, drugs, bacterial infection and intoxication). By these stimuli (i.e. stressors), stress is generally produced as the sum reactions in the body. In general, marked variations are found in percentages of biological response of different kinds occurring in the bodies, depending on the intensity of stressor and the duration of exposure as well as the individual susceptibility. There seem to be no general mathematical equations in the literature (Chung, 1959, 1960, 2011), to my knowledge that express a quantitative relationship among three factors, namely the intensity of stressor, the duration of exposure and percentage of occurrence of a response in biological phenomena. The author presented a general formula, **Eq. 1** on the basis of animal experiments carried out by the author and his coworkers (Chung, 1960; Kim and Chung, 1962).

$$P = [(i - a)\, t^{n} - c] / (b\, t^{n} + d) \quad (1a)$$

$$Q = \frac{10}{\sqrt{(2\pi)}} \int_{-\infty}^{P} \exp[-(P - 50)^2/200]\, dP \quad (1b)$$

where

i = intensity of stressor (stimulus) t = duration of exposure to stressor *a, b, c, d* and *n* are constants.
P = 'probacent' (abbreviation of probability percentage).
Q= probability in percentage of occurrence of response.

The 'probacent' is associated with and a function of two independent variables. The 'probacent' values of 0, 50 and 100 correspond to (mean-5 SD), mean and (mean+5 SD) (SD: standard deviation) as described in the author's previous publications (Chung, 1960, 2013, 2018b). The unit of 'probacent' is 0.1 SD.

Eq. 1 is applicable to biological phenomena in which Gaussian normal distribution is applicable such as tolerance of animals to chemicals, heat, anesthetics, carbon monoxide (Chung, 1960).

Eq. 2 is applicable to biological phenomena such as drug tolerance and electroshockconvulsion in mice in which lognormal distribution is applicable.

A general formula, **Eq. 2** was derived to express a mathematical relationship among the drug dose, the time after administration, and the percentage of a response in animals (Chung, 1960, 1986).

$$P = 100 \cdot [\log D - \log (a + c/t^{n})] / [\log ((a + 100.b + (c + 100.d)/t^{n})) - \log (a + c/t^{n})] \quad (2a)$$

$$Q = \frac{10}{\sqrt{(2\pi)}} \int_{-\infty}^{P} \exp[-(P - 50)^2/200]\, dP \quad (2b)$$

Where

D = dose of administered drug t = time after administration of drug *a, b, c, d* and *n* are constants
P = modified 'probacent' and a function of D and t.

The author published **Eq. 3** that expresses the mortality of mice administered Metrazol as a function of the dose and time after administration (Chung, 1960, 1986).

The Compaq Presario Windows 95 that the author had used for computer computation with UBASIC suddenly stopped to work in June 2017. The author examined a possibility of Calcline program, employing Apple computer, and found a complete agreement between the results of Calcline- and UBASIC- derived solid cancer (Q), leukemia (R) and radiation-exposureinduced-death (REID) for astronauts in future space flight to Mars in the author's comparative study (Chung, 2018a, 2019).

In this study, Calcline program is applied to construct the 'probacent ' formulas to express tolerance of mice to Metrazol and electroshock. The method how to construct the 'probacent' formula in biomedical research is particularly described in detail.

2. Material

Chung (1986) published a general formula of the 'probacent'-probability equation that expresses the tolerance of mice to Metrazol, and that expresses the mathematical relationship among the drug dose of Metrazol, the time after administration and the biological response of respiratory arrest as a sign of mortality in mice by use of a microcomputer program (Chung, 2009).

The electroconvulsive therapy (ECT) is used in the shock treatment of certain psychiatric patients. In the literature, to my knowledge. there seem to be no general mathematical equations that express a quantitative relationship among voltage of electroshock, the duration, and the percentage of occurrence of convulsion in man or animals (Weiner, 1985; Crowe,1984; Karasu, 1984; Takao, 1958; Turner,2019).

Chung (1989c) reported a microcomputer program in BASIC for predicting percentage of occurrence of convulsion in mice administered electroshock, and published a formula that expresses the mathematical relationship among the voltage of electroshock, the duration and the percentage of occurrence of convulsion in mice.

The results of the above two articles are used in this study to examine applicability of the computer program, Calcline, employing Apple computer, MacBook OS-X.

3. Method

3. 1. Tolerance of mice to Metrazol

150 white mice weighing 17-25 g were used in the coworker and author's study (Hur and Chung, 1962) to determine tolerance of mice to Metrazol (Knoll Pharmaceutical Company, Whippany, NJ, USA), a central nervous system stimulant. Metrazol was subcutaneously injected to the back of mice. After injection of various doses of Metrazol, mice were observed with regard to onset of respiratory arrest as a sign of mortality at different times. The experimental results were analyzed (Hur and Chung, 1962). The data of mortality and **Eq. 3** were used in this study to design a computer program. **Eq. 3** is applicable to tolerance of mice to Metrazol (Chung, 1960, 1986).

$$P = 100 \cdot [\log D - \log (0.1 + 2.61/t^{1.455})]/[\log (5.5 + 173.61/t^{1.455}) - \log (0.1 + 2.61/t^{1.455})] \qquad (3a)$$

$$Q = \frac{10}{\sqrt{(2\pi)}} \int_{-\infty}^{p} \exp [- (P - 50)^2 /200] \, dP \qquad (3b)$$

where t = time in minutes after administration of Metrazol Q = predicted mortality of mice in percentage.

A computer program was written for a microcomputer (Chung, 2009). Instead of the integral formula (Hastings, 1955):

$$ø(X) = 2/\sqrt{\pi} \int_0^x e^{(-t^2)} \, dt \qquad (4)$$

$$ø(X) = 1 - 1/ (1 + A_1.X + A_2.X^2 + A_3.X^3 + A_4.X^4)^4 \qquad (5)$$

$A_1 = 0.278393$
A_2 = o.230389
$A_3 = 0.000972$ $A_4 = 0.078108$

For transformation of Eq. 3b to Eq.4:

$t = (P - 50)/\sqrt{200}$ $dt = dP/\sqrt{200}$
$X = (P - 50)/\sqrt{200}$ (6) If $(P - 50) < 0$:
$Q = 50/(1+ A_1.X + A_2.X^2 + A_3.X^3 + A_4.X^4)^4$ (7) If $(P - 50) \geq 0$:
$Q = 50 ø (\infty) + 50 ø [(50 - P)/\sqrt{200}]$
$Q = 100 - 50 / (1 + A_1.X + A_2.X^2 + A_3.X^3 + A_4.X^4)^4$ (8)

Eqs. 6, 7 and 8 were used in the BASIC computer program.

3. 1. 1. Geometric graphical analysis of data for construction of the 'probacent' Eq. 3a prior to computation of the integral Eq. 3b.

Various doses of drug, Metrazol were given to animals by a certain mode of administration. Thereafter, percentages of occurrence of certain response, respiratory arrest as a sign of mortality were measured at various given times (**Table 1**). Results are plotted on a log-log graph paper. Doses of drugs are taken along the ordinate and time after administration along the abscissa (**Figure 1**). If points indicating 50 % responses at each dosage level are connected, they reveal a rectilinear line with a definite declination (θ) at higher doses. Three lines indicating specific percentages of occurrence of response, e.g. 0, 50 and 100%, may be likewise parallel to each other at higher doses.

The value of the constant n in **Eq. 3a** relating to 'probacent' can be obtained from the declination (θ) as shown in **Fig. 1** (Chung, 1960, 1986) as follows: $n = \tan \theta$
For instance, the declination of the line of 50% response to Metraziol reveals 55° 30', so the value of n is:
$n = \tan 55° 30' = 1.455$

The value of the constant a in Eq.3a can be obtained from LD_0 at the infinite time, that represents the asymptote along the abscissa in **Fig. 1**. Substituting $t = \infty$ and $P = 0$ in **Eq.3a,** following equation is derived:
$a = D$.

The above described LD_0 may be determined graphically as shown in **Figure 2** for Metrazol. Results of the longest period of observation, e.g. 1440 min for Metrazol, are plotted on the probacent- probability graph paper. Doses are taken along the abscissa of logarithmic scale giving LD_0 = 0.1 mg/10g body weight and $a = LD_0$ = o.1.

The value of the constant c in **Eq. 2a** can be calculated from one set of data with a condition of $P = 0$, by substituting values of $D, t, P, n,$ and a in **Eq. 2a**. For example, with D = 10mg/10g body weight, t = 0.4 min, $P = 0$, $n = 1.455$, and a = 0.1 (the value of 0.4 min is determined graphically from the probability paper as shown **in Fig. 2**), $c = 2.61$.

The values of the constants b and d in **Eq. 2a** can be determined from two sets of data by substituting values of D, t, P, n, a and c in **Eq. 2a.**

For example:

(1) D = 25 mg/10 g body weight, t = 4.5 min, $P = 100$, $n = 1.455$, $a = 0.1$ and c =2.61.

(2) *D* = 10 mg/10 g body weight, *t* = 12.3 min, *P* = 100, *n* = 1.455, *a* = *o.1*, and c = 2.61 The values of 4.5 min for the dose of 25 mg and 12.3 min for the dose of 10 are determined graphically from the probability paper as shown in **Figure** 2. Values of *b* and *d* are calculated from the equations derived from **Eq. 2a** as: *b* = 0.054 and *d* =1.71.

Table 1. Tolerance of mice to subcutaneously administered Metrazol, determined by onset of respiratory arrest (mortality).

Metrazol dose (Mg/10g body weight)	Time after injection (min)	No. of animal's (*M*)	No. dead (*N*)	Actual % mortality	Computer-derived % (Pa)	Probacent (Q) (P)
25	0.5	5	0	0	2	29.48
	0.75	5	1	20	25.1	45.29
	1	5	2	40	61.7	52.97
	1.5	5	4	80	95	66.37
	2	5	5	100	99.5	75.61
	0.5	10	0	0	2	29.48
	0.7	10	1	10	18.3	40.95
	1	10	4	40	61.7	52.97
	1.5	10	9	90	95	66.37
	2.5	10	10	100	99.9	82.6
	0.5	10	0	0	2	29.48
	0.7	10	1	10	18.3	40.95
	1	10	3	30	81.7	52.97
	1.5	10	7	70	95	66.37
	2	10	10	100	99.5	75.61
10	1	10	0	0	3	31.13
	2	10	3	30	61	53.75
	2.5	10	5	50	85.7	60.68
	3	10	9	90	95	66.16
	4.5	10	10	100	99.7	77.55
5	0.5	20	0	0	0	-8.87
	1	20	2	10	0	14.6
	2.5	20	4	20	27.8	44.13
	3.5	20	9	45	65.7	54.05
	4	20	12	60	78.2	57.78
	5	20	17	85	91.4	63.68
	6	20	19	95	96.6	68.16
	8	20	20	100	99.3	74.51
3	2	10	0	0	0.6	25.01
	3	10	1	10	10.4	37.38
	5	10	4	40	55.7	51.43
	6	10	7	70	72.2	55.89
	8	10	9	90	88.9	62.21
	9	10	10	100	92.7	64.52
2.5	3	20	0	0	4.5	33.03
	4	20	1	5	18.9	41.19
	5	20	5	25	38.4	47.06
	7	20	9	45	69.2	55
	8	20	13	65	78.3	57.81
	10	20	17	85	88.6	62.05
	13	20	19	95	94.8	66.28
	17	20	20	100	98	69.81
1	5	14	0	0	0.7	25.1
	10	14	1	7.1	15.7	39.92
	15	14	5	35.7	34.4	45.99
	20	14	7	50	46.8	49.2
	25	14	8	57.1	54.5	51.14
	30	14	9	64.3	59.6	52.43
	80	14	10	71.4	73	56.11
	1440	14	10	71.4	77.2	57.44

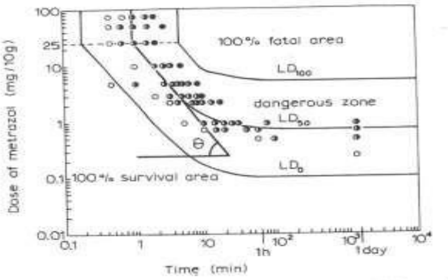

Fig. 1. Tolerance of mice to subcutaneously administered metrazol, determined by onset of respiratory arrest (mortality). Abscissa: time in minutes after injection of metrazol. Ordinate: dose in mg/10 g body weight of metrazol. A log–log scale is used. A closed circle indicates a 100% actual mortality point. A half closed circle indicates an actual mortality point between 0 and 100%. An open circle indicates a 0% actual mortality point. The value of the constant n in Eq. 3a relating to 'probacent' (see the text) can be determined from the declination (θ) of the line indicating the 50% response (mortality), i.e. LD_{50} at higher doses. $n = \tan\theta = \tan 55° \ 30' = 1.455$. The dashed line indicates Dm (25 mg/10 g).

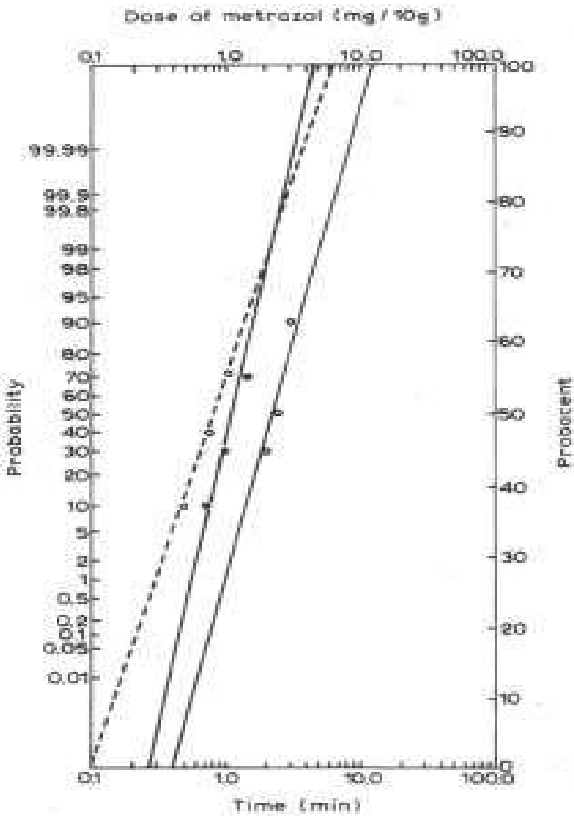

Fig.2 . Results of tolerance of mice to metrazol are plotted on a probability–probacent semi-log graph paper. The ordinate represents percent probability (Q) of response (mortality) on the left scale, and the corresponding 'probacent' (P) on the right scale. The dashed line connects points of data observed at 1440 min after injection of metrazol. Doses of metrazol are taken along the abscissa. The two solid lines connect points of data observed with the doses of 25 mg (closed circles) and 10 mg (open circles) of metrazol. Time after injection is taken along the abscissa.

3. 2. Tolerance of mice to electroshock

120 adult mice weighing 16-29 g were used in the coworkers and author's study (Park and Chung, 1961) to investigate a possibility of expressing the percentage of occurrence of convulsion in mice as a function of the stimulus voltage and the duration. An electrical stimulus was applied to the shaved skin of bilateral preauricular frontal region of a mouse fixed in a prone position on a Bakelite plate.

Eq. 9 is applicable to express tolerance of mice to electroshock (Chung, 1989c) as shown in **Table 2**, and F**igures 3 and 4**.

P = 100 x [log V - log (0.5 + 7.375/t)]/[log (32.4 + 165.275/t) - log (0.5 + 7.375/t)] (9a)

$$Q = \frac{10}{\sqrt{(2\pi)}} \int_{-\infty}^{P} \exp\left[-\,(P-50)^2/200\right] dP \qquad (9b)$$

where

V = stimulus voltage in volts, t = duration in seconds,

P = modified 'probacent',

Q = predicted percent probability of occurrence of convulsion in mice.

Table 2. Relationship among voltage of electroshock, duration of current, and percentage **Of occurrence of convulsion in mice.**

Voltage (V)	Duration (s)	No. of animals	No. of convulsion	Actual % convulsion (Pa)	Computer-derived % (Q)	Probacent (P)
100	0.5	7	0	0	0.9	26.21
	1	7	2	28.6	35.5	46.283
	1.5	7	5	71.4	76	57.052
7 7 100 92.1 64.085						
7 7 100 98.9 72.949						
80 0.5 8 0 0 0.9 26.21 1 8 2 25 35.5 46.283						
	1.5	8	7	87.5	76	57.052
8 8 100 92.1 64.085						
8 8 100 98.9 72.949						
50	0.5	11	0	0	0.9	26.21
	1	11	1	9.1	35.5	46.283
1.5 11 7 63.6 76 57.052 2 11 10 90.9 92.1 64.085						
	2.5	11	11	100	97.2	69.127
	3	11	11	100	98.9	72.949
30	0.5	21	0	0	0.2	21.347
	1	21	1	4.8	19.8	41.5
1.5 21 12 57.1 59.2 52.339 2 21 18 85.7 82.7						
59.433 2.5 21 20 95.2 92.7 64.528						
3.5 21 21 100 98.4 74.451 20 0.5 21 0 0 0 8.555 1.5 21 2 9.5 15.7 39.941						
	2.5	21	12	57.1	59.6	52.431
	4	21	20	95.2	88.8	62.171
	8	21	21	100	98.9	73.043
10	1	22	0	0	0.2	7.412
6 22 12 54.5 48 49.494 8 22 14 63.6 65.2						
53.893						
	10	22	17	77.3	75.3	56.822
	15	22	18	81.8	86.7	61.148
	20	22	20	90.9	91.2	63.527
	30	22	22	100	94.6	66.078
5	2	20	0	0	0	5.352
	15	20	6	30	23.6	42.808
	20	20	7	35	32.6	45.505
	30	20	8	40	43.8	48.434
	40	20	10	50	50	44.999
	60	20	11	55	56.5	51.642
	100	20	12	60	61.9	53.019
	180	20	12	60	65.5	53.97
1	180	0	0	0	0	14.911

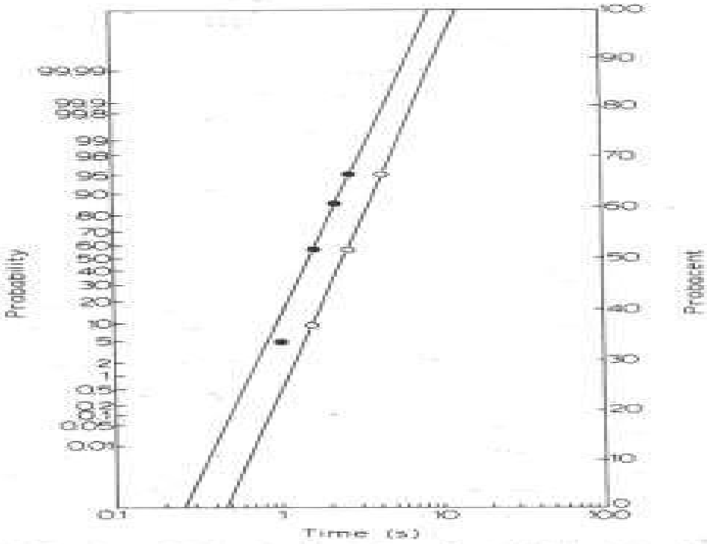

Fig.4 Results of electrically induced convulsion in mice are plotted on a probability–probacent semi-log graph paper. The ordinate represents percent probability (Q) of convulsion on the left scale, and the corresponding 'probacent' on the right scale. Time is taken along the abscissa of log scale. The two solid lines connect points of data observed with the voltages of 30 V (closed circles) and 20 V (open circles).

3. 2. 1. Geometric graphical analysis of data for construction of the 'probacent' Eq. 9a prior to computation of the integral Eq. 9b.

Experimental results are plotted on a log-log graph paper (**Figure 3**). Voltages of electroshock are taken along the ordinate and duration along the abscissa. The values of 50% effective voltage (EV_{50}) for 30 and 20 V levels can be determined from the straight lines connecting plotted points for each voltage in **Figure 4** that shows relatively higher levels of electroshock are plotted on a probability-probacent semi-log graph paper. EV_{50} values are 1.37 and 2.35 s for 30 and 20 V, respectively. These values are used for determination of the declination (θ) by connecting the EV_{50} points in **Figure 3**. If the two points indicating 50% convulsion response at 30 and 20 V levels are connected in **Figure 3**, they reveal a line with a decllnation (θ) at these higher levels the declination (θ) is mesured as 45^0.

The value of the constant n in **Eq. 2a** ($D = V$ in **Eq. 2a**) relating to 'probacent' can be measured from the declination as shown in **Figure 3** (Chung, 1986, 1989a; Park and Chung, 1961) as follows:

$n = \tan\theta = \tan 45^0 = 1$

The value of the constant a **in Eq. 2a** can be obtained from EV_0 (0% effective voltage) at the infinite time, that represents the asymptote along the abscissa in **Figure 3**. Substituting $t = \infty$ and $P = 0$ in **Eq. 2a**, the following equation is derived:

$a = V$

Since only one set of results with the condition of $P > 0$ and the longer duration of 180 s is available in the data (**Table 2**), graphical analysis with the probability-probacent graph paper (**Figure 4**) cannot be done to approximately determine EV_0. Consequently, the value of EV_0 is assumed to be 0.5 V from the experimental data:

$a = 0.5$

The value of the constant c **in Eq. 9a** can be calculated from one set of data with a condition of $P = 0$ by substituting values of V, t, P, n and a in **Eq. 2a**. With the following data, $V = 30$ V, $t =$o.25 s, $P =$ o, $n = 1$, $a = 0.5$ (the value of 0.25 s is determined graphically from the probability-probacent graph paper as shown in **Figure 4**.

$c = 7.375$

The values of the constants *b* and *d* **in Eq.2a** can be determined from two sets of data by substituting values of *V, t, P, n, a* and *c* in **Eq. 2a**.

(1) *V* = 30 V, *t* = 1.37 s, *P* = 50, *n* = 1, *a* = 0.5, and *c* = 7.375;

(2) *V* = 5 *V, t* = 40 *s,* P = 50, *n* = 1, *a* = 0.5, and *c* = 7.375; (these values of 5 V, 40 s, and 50 are taken from actually observed data shown in **Table 2**). The value of 1.37 s for 30 V is determined graphically as above described from the probability-probacent paper as shown in **Figure 4**. Values of *b* and *d* are calculated from **Eq. 2a**:

b = 0.319 and *d* = 1.579

Eq. 9a of 'probacent' is finally established.

3. 3. Computation of Eqs. 3b and 9b.

Instruction of Calcline program is to be followed to carry out computer computation of the approximation equations **Eqs. 4** and **5** instead of the integral **Eqs. 3b** and **9b**.

3. 4. Description of BASIC computer program

A BASIC computer program was written for an IBM PC microcomputer (International Business Machines Corporation, Boca Raton, FL) and compatibles. The BASIC program **for Eq. 9b** that expresses mortality probabilities (*Q*) in mice exposed to electroshock uses a formula of approximation (Hastings, 1955; Chung, 1986). Values of probacent *(P)* and mortality probability (*Q*) are calculated (Chung, 1989).

BASIC COMPUTER PROGRAM FOR CALCULATING PROBACENT (P) OF EQ. 3a AND PERCENTAGE OF OCCURRENCE OF CONVULSION (Q) OF EQ. 3b IN MICE EXPOSED TO ELECTROSHOCK (CHUNG, 1989)

```
10 LPRINT "RELATIONSHIP AMONG ELECTROSHOCK VOLTAGE, DURATION OF
CURRENT, AND PERCENTAGE OF OCCURRENCE OF CONVULSION IN MICE" 20 LPRINT
30 LPRINT
"VOLTAGE";TAB(10);"DURATION";TAB(20);"NO.OF";TAB(42);"%";TAB(54);COMPU
TER"; TAB(65);"PROBACENT"
40 LPRINT
"VOLT";TAB(10)"SEC";TAB(20);"ANIMALS";TAB(30);"CONVULSION"TAB(42);CON
VULSION";TAB(54);"DERIVED";TAB(65);"%"
50 LPRINT TAB(54);"PERCENTAGE"
60 LPRINT TAB(20);"(M)";TAB(30);"(N)";TAB(42);"(Pa)";TAB(54);"(Q)";TAB(65);"(P)'
70 READ V,T,M,N
80 IF V>=35 THEN V=35 ELSE V =V
90 FNP(V,T)=100*(LOG(V)/LOG(10)-LOG(.5+7.375/T)/LOG(10))/(LOG(32.4+165.275/T)LOG(.5+7.375)/T))
100 A1=.278393
110 A2=.230389
120 A3=.000972
130 A4=.078108
140 IF (FNP(V,T)-50)<0 THEN 150 ELSE 180
150 X=(FNP(V,T)-50)/SQR(200)
160 Q=50/(1+A1*X+A2*X^2+A3*X^3+A4*X^4)^4
170 GOTO 200
180 X=(FNP(V,T)-50)/SQR(200)
190 Q=100-50/(1+A1*X+A2*X^2+A3*X^3+A4*X^4)^4
200 LPRINT
TAB(3);V;TAB(13);T;TAB(23);M;TAB(33);N;TAB(42);CINT(10*M.N*100)/10;TAB(54);
CINT(10*Q)/10;TAB(65);CINT(10^2*FNP(V,T))/10^2
210 GOTO 70
220 DATA 100,0.5,7,0.100,1.0,7,2,100,1.5,7,5,100,2.0,7,7,100,3.0,7,7,80,0.5,8,0
230 DATA 80,1.5,8,7,80,2.0,8,8,80,3.0,8,8,50,0.5,11,0,50,1.0,11,1,50,1.5,11,7,50,2.0,11,10
240 DATA
50,2.5,11,11,50,3.0,11,11,30,0.5,21,0,30,1.0,21,1,30,1.5,21,12,30,2.0,21,18,30,2.5,21,20
250 DATA
30,3.5,21,21,20,0.5,21,0,20,1.5,21,2,20,2.5,21,12,20,4.0,21,20,20,8.0,21,21,10,1.0,22,0
260 DATA 10,
6.0,22,12,10,8.0,22,14,10,10.0,22,17,10,15.0,22,18,10,20.0,22,20,10,30.0,22,22
270 DATA 5,2.0,20,0,5,15.0,
20,6,5,20.0,20,7,5,30.0,20,8,5,40.0,20,10,5,60.0,20,11,5,100.0,20,12,1,5,180.0,20,12,1,180.0, 10,0
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3. 5. Statistical analysis

A chi square goodness-of-fit test (Dixon and Massey, 1957) is used to test fit of a model to the observed data. The difference is statistically significant if the $p < 0.05$.

4. Results

4. 1. Tolerance of mice to Metrazol
Table 1 shows the actual (P_a), the computer-assisted, Calcline-derived mortality (Q) and the probacent (P) in relation to the Metrazol dose (D), the time (t) after administration.

The chi square goodness-of-fit test reveals a remarkable agreement between the actual mortality (P_a) and the computer-assisted, Calcline-derived mortality (Q) (p >0.995).

Figure 1 illustrates tolerance of mice to subcutaneously administered Metrazol, determined by respiratory arrest, mortality.

4. 1. 1. Calcline calculation
Dose=25 mg/10 g, time (t) after administration=0.5 min.
P=100*(log (25)/log (10) - log (0.1+2.61/0.5^1.455)/log (10))/ (log (5.5+173.61/0.5^1.455)/log (10) - log (0.1+2.61/0.5^1.455)/log (10))
=29.48979
X= (50 - 29.9803)/200^(1/2)
=1.45029
Q=50/(1+0.278393*1.45048+0.230389*1.45048^2+0.000972*1.45048^3+0.078108*1.45048
^4
)^4
=1.99719

The other P and Q values in relation to various Metrazol doses (D) and time (t) are obtained similarly to the above Calcline calculation.

4. 2. Tolerance of mice to electroshock
Table 2 shows the actual percentage (P_a), the computer-assisted, Calcline-derived predicted percentage of convulsion(Q) in relation to the voltage and duration of electroshock.

Figure 3 illustrates the relationship among the voltage of electroshock, the duration and the percentage of occurrence of convulsion in mice.

4. 2. 1. Calcline calculation
Voltage (V)=100 V, duration (t)=0.5 second.
P=100*(log (100)/log (10)-log (0.5+7.375/0.5)/log (10))/ (log (32.4+165.275/0.5)/log (10) - log (0.5+7.375/0.5)/log (10))
=26.21
X= (50-26.21)/200^(1/2)
=1.682212
Q=50/(1+0.278393*1.68221+0.230389*1.68221+0.000972*1.68221^2+0.000972*1.68221^3 +0.08108*1.68221^4)^4
=0.87376

The other P and Q values in relation to various voltages (V) and duration (t) are obtained similarly to the above Calcline calculation.

5. Discussion
The Gaussian form concerning the normal frequency curve is generally used to represent distributions of animals with regard to one single variable in biostatistics. A basic formula (**Eq. 2a**) of 'probacent' (P) is a function of two variables; the intensity (i) of stressor (stimulus) and duration (t) of exposure to stressor. The general formula (**Eq. 2**) is used to represent a probability of certain response in animals by incorporating 'probacent' in the formula of an integral of the Gaussian normal distribution curve. Therefore, the Gaussian form of the normal frequency curve can be used in predicting probability in biological phenomena with two variables of intensity and duration of stressor. This study presents a probability model of a bivariate normal distribution for a pair of continuous random variables (dose and time) (Lindgren, 1975). The just-effective dose of drugs and poisons is often distributed lognormally (Bliss, 1967; Chan, 1982). **Eq. 3a** is a function of log dose D and time T.

The chi square goodness-of-fit test reveals a remarkable agreement between the actual mortality and the computer-assisted Calcline-derived predicted mortality and a fair accuracy (p> 0.995).

The original equation of Park and Chung (1961) derived from the experimental data was based on the general **Eq. 1**. The equation approximately expressed the relationship among the voltage, the duration, and the percentages of occurrence of convulsion in mice. The data and the equation of Park and Chung were reviewed and reanalyzed in this study. Analysis reveals that the voltage of electroshock or the duration appears to be distributed lognormally with regard to induction of convulsion as described in Methods. Therefore, the general **Eq. 2** is applied to more closely express the above described relationship (Chung, 1960, 1986). The chi square goodness-of-fit test reveals a remarkable agreement between the actual, and computer-assisted, Calcline-derived percentages of occurrence of convulsion in mice ($p > 0.995$).

The basic formula **Eq. 1a** was applied to fairly accurately express to carboxyhemoglobin levels of blood as a function of carbon monoxide concentration in air and duration of exposure (Chung, 1988). The probacent model has been applied to data in biomedical literature to express a relationship among plasma acetaminophen concentration, time after ingestion and occurrence of hepatotoxicity in man (Chung, 1989a); express survival probability in patients with heart transplantation (Chung, 1993); to express survival probability in patients with chronic leukemia, acute myelogenous leukemia or malignant melanoma (Chung, 1989b, 1991b, 1994a); to predict the percentile of serum cholesterol levels by age in adults (Chung, 1990); to express a relationship among age, height and weight, and percentile in Saudi and US children of ages 6-16 years (Chung 1994b); to predict the percentile of heart weight by body weight in subjects from birth to 19 years of age (Chung, 1990); to express the age-specific survival probability, death rate and life expectancy in US adults, men and women (Chung, 1995, 2007); to express cancer mortality risk after exposure to acute low dose ionizing radiation in humans (Chung, 2012, 2013, 2017); to express radiation safety for astronauts in future space flight to Mars (Chung, 2018a).

A 'probacent' forumula (*P)*, **Eq.10** is also applicable to age-specific (*t*) survival probability (*S*), life expectancy and death rate of human populations, and tolerance of humans to ionizing radiation (*D*). **Eq. 10** can be derivable from **Eq. 1** (Chung, 2012).

$P^r = A - B \log (t)$ or (D) (10a)

$S = \frac{10}{\sqrt{(2\pi)}} \int_{-\infty}^{P} \exp[-(P-50)^2/200]\, dP$ (10b) where t = age

D = dose of radiation *A, B* and *r* are constants.
P = probacent
S = survival probability (%).

The probacent formula was applied to express survival probability as a function of inoculum size of leukemia cells and time after inoculation in mice (Chung, 1991a).

Mathematical laws are believed to be applicable to the physical world of the universe (Newton, Hawking). Einstein's theory of relativity regarding the relation between energy (E) and mass (m): $E=mc^2$ is applicable to quantum mechanics. The 'probacent' formula seems to be likewise applicable to biological phenomena. It may be believable that "all our life is governed with mathematical precision by God's intelligibly framed cosmic laws" (Yogananda, 2006).

The purpose of the research on the 'probacent' formula is that the formula would be hopefully helpful to prevention and treatment of humans or patients who are exposed to harmful circumstances or noxious agents.

6. Conclusion

On the basis of results of this study**,** the following conclusion is proposed**.**

[1]. A computer program of Calcline can be used to construct the general formula that expresses mortality (tolerance) of mice as a function of the Metrazol dose and the time after administration.

[2]. A remarkable agreement is present between the actual, and the computer-assisted predicted mortality values in tolerance of mice to Metrazol ($p > 0.995$).

[3]. A computer program of Calacline can be used to construct the general formula that expresses percentages of occurrence of convulsion as a function of the the voltage and the duration of electroshock.

[4]. A remarkable agreement is present between the actual, and computer-assisted predicted percentages of occurrence of convulsion in tolerance of mice to electroshock ($p > 0.995$).

[5]. It has been found that Apple computer, MacBook with application of Calcline program can be similarly used to construct general 'probacent' formulas that express mathematical relationship among intensity of stimulus such as Metrazol and electroshock, duration of exposure and percentage of occurrence of response in animals in biological phenomena as BASIC and UBASIC programs were successfully used in case of employing Windows 95 in mathematical approach in biomedical research.

[6]. The method how to construct general 'probacent' formulas in mathematical approach in biological-phenomena research is described in detail in this study.

[7]. The purpose of the research on the 'probacent' formula is that the formula would be hopefully helpful to prevention and treatment of humans or patients who are exposed to harmful circumstances or noxious agents.

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