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ANALYSIS OF QUANTITATIVE DATA OBTAINED FROM TOXICITY STUDIES SHOWING NON-NORMAL DISTRIBUTION

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ABSTRACT — The data obtained from toxicity studies are examined for homogeneity of variance, but, usually, they are not examined for normal distribution. In this study I examined the measured items of a carcinogenicity/chronic toxicity study with rats for both homogeneity of variance and normal distribution. It was observed that a lot of hematology and biochemistry items showed non-normal distribution. For testing normal distribution of the data obtained from toxicity studies, the data of the concurrent control group may be examined, and for the data that show a non-normal distribution, non-parametric tests with robustness may be applied.

KEY WORDS: Shapiro-Wilk test, Normal distribution, Toxicity study, Homogeneity of variance

INTRODUCTION

Several statistical techniques, for example the *t*-test and variance analysis, are based on the assumption that the data show a normal distribution and homogeneity of variance. Before applying any statistical techniques, the data are examined for homogeneity of variance (Yoshimura, *et al*., 1992). To check the homogeneity of variance of data, Bartlett's test is commonly used. When the data are found to be homogeneous of variance, parametric tests like Dunnett multiple comparison test or Williams test are used, whereas for the data of heterogeneous variance, a non-parametric test (rank sum test) is used. But, most of the results obtained from the toxicity studies do not show a normal distribution. In an NTP report (1997) two approaches were employed to assess the significance of pair-wise comparisons between exposed and control groups in the analysis of continuous variable. Organ weight and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparisons procedures of Dunnett and Williams. Hematology, clinical chemistry, spermatids, and epididymal spermatozoa, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison method of Shirley and Dunn. In this study, I identified the measured items obtained from a carcinogenicity/chronic toxicity study with rats that showed a non-normal distribution and suggested how to deal with such kinds of items.

MATERIALS AND METHODS

For data showing normal distribution, Kolmogorov-Smirnov, Lilliefors, Shapiro-Wilk tests, the tests for goodness of fit by the Chi-squared distribution, etc. are generally used (Muto, 2000). In the present study, Shapiro-Wilk test (Shapiro and Wilk, 1965, JMP version 5, SAS, USA) was employed.

Statistical analysis

Data obtained from a carcinogenicity/chronic toxicity study in F344 male rats carried out at An-Pyo Center, Shizuoka, Japan, were considered here. The data of concurrent control group were analyzed by Shapiro-Wilk test (Shapiro and Wilk, 1965) for normality. The skewness and kurtosis of various parameters were calculated to investigate the indicator to the variance. The homogeneity of variance was determined using Bartlett's test (Gad and Weil, 1986).

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RESULTS

The data for males of the concurrent control group of the above study were analyzed by Shapiro-Wilk test for normality. The effect of number of samples on the power of Shapiro-Wilk test for normality is given in Table 1. The power increased as the number of samples increased.

The measured items, which showed a non-normal distribution were food consumption, hematocrit, hemoglobin, red blood cells, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (gamma-GTP), potassium and free cholesterol. Additionally, absolute spleen weight, and absolute weight/body weight ratio of heart, spleen and adrenal glands also showed a non-normal distribution. It was observed that on Week 104, white blood cells (WBC), protein, glucose, triglyceride, total cholesterol, free cholesterol, NEFA, phospholipid, chloride, absolute heart, liver and adrenal weights, and absolute weight/body weight of brain and liver did not show normal distribution. Body weight, clinical test values and organ weights other than the above-mentioned almost showed the normal distribution (Table 2).

The items to skew to the right distribution with sharp kurtosis by non-normal distribution were the MCV, MCH, platelet, AST, ALT, ALP, Gamma-GTP, creatinine phosphokinase (CPK), protein, free cholesterol, potassium and absolute spleen weight and absolute weight/body weight ratio of heart, spleen and adrenal gland values. The items to skew to the left distribution with sharp kurtosis by non-normal distribution were the food consumption, hematocrit, hemoglobin, red blood cell and MCHC (Table 3).

Measured parameters that showed non-normality at different weeks are given in Table 4. It is obvious from the table that, on Week 104, more number of measured items showed non-normal distribution compared to Weeks 26, 52 or 72.

DISCUSSION

The test for homogeneity of variance is first executed by the Bartlett test in toxicity studies. But this test does not indicate that the data follow a normal distribution. In the present study, the quantitative items of F344 male rats of the concurrent control group of the carcinogenicity/chronic toxicity study were investigated. For the analysis of normal distribution, Shapiro-Wilk test was used. It is generally considered that red blood cell, white blood cell, platelets etc., usually show normal distribution and enzyme activities such as ALT, AST etc., show a non-normal distribution. On the contrary, in the present study, these measured items along with several others showed a non-normal distribution. The measured items in which a normal distribution was not secured were food consumption, hematocrit, hemoglobin, red blood cell, MCV, MCH, MCHC and platelet values AST, ALT, ALP and Gamma-GTP, potassium, free cholesterol, and absolute spleen weight, and weight/body weight ratio of heart, spleen and adrenal glands.

The majority of scientific papers discuss only testing homogeneity of variance and not testing normal distribution of the data. The data that show homogeneity of variance necessarily do not show a normal distri-

Table 1. Effect of number of samples on the power of Shapiro-Wilk test for normality in body weight gain of F344 rats.

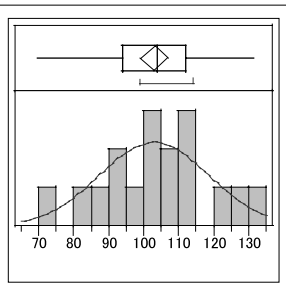
No. of sample	Histogram	Mean	Coefficient of variance (%)	Shapiro-Wilk test for normality	
				W value	*P* (Prob<W)
17		103	15.5	0.987278	0.9891
34			15.3	0.968746	0.5017
51			15.2	0.959888	0.1486
68	N=68		15.2	0.954862	0.0383

Table 2. Normality to quantitative data obtained in toxicological study of control male F344 rats analyzed by the Shapiro-Wilk test.

Measured items	Week after dosing	Mean ± S.D. (N)	Skewness	Kurtosis	*W* value	*P* (Prob<W)
Body weight (g)	1	115 ± 7 (80)	0.198776	0.368405	0.983653	0.7634
	13	295 ± 16 (80)	–0.185918	0.193111	0.990349	0.9682
	26	344 ± 17 (80)	0.057970	0.302906	0.992462	0.9901
	52	285 ± 20 (69)	0.262169	0.019957	0.978060	0.5499
	78	415 ± 27 (58)	0.189785	0.103944	0.979552	0.6684
	104	391 ± 22 (40)	0.432809	–0.665561	0.950462	0.1121
Food Consumption (g/week)	1	90 ± 5 (80)	–0.132049	–0.081082	0.976344	0.4244
	13	106 ± 8 (80)	–0.844292	3.429470	0.958118	0.0359
	26	113 ± 7 (80)	–0.386683	0.766378	0.979103	0.5497
	52	116 ± 8 (80)	–1.287890	5.895770	0.932610	0.0004
	78	116 ± 8 (80)	0.466967	0.348639	0.959531	0.0450
	104	121 ± 11 (80)	0.836442	1.096030	0.933861	0.0005
Hematocrit (%)	26	45.2 ± 0.6 (10)	–0.479387	–0.287092	0.971223	0.8939
	52	44.4 ± 6.7 (10)	–2.860395	8.637416	0.580580	<0.0001
	78	43.7 ± 11.1 (10)	–1.709545	3.493266	0.780651	0.0088
	104	47.6 ± 4.3 (38)	–1.545748	4.727300	0.900994	0.0025
Hemoglobin (g/dL)	26	15.5 ± 0.2 (10)	0.274740	–0.726034	0.954148	0.7030
	52	14.4 ± 2.8 (10)	–3.001727	9.249612	0.511151	<0.0001
	78	14.7 ± 1.6 (10)	–2.748375	8.237457	0.603210	<0.0001
	104	15.3 ± 1.4 (38)	–1.618717	4.794731	0.885137	0.0007
Red blood cell ($\times 10^6$/mm^3)	26	9.15 ± 0.12 (10)	–1.341302	1.110607	0.841765	0.0450
	52	8.68 ± 1.51 (10)	–3.080247	9.961014	0.473240	<0.0001
	78	8.65 ± 1.24 (10)	–2.292873	8.993870	0.548117	<0.0001
	104	8.72 ± 1.10 (38)	–2.183697	7.440757	0.827761	<0.0001
MCV (μm^3)	26	49.4 ± 0.4 (10)	0.327506	–0.133513	0.937067	0.5044
	52	51.5 ± 2.8 (10)	2.286761	6.419465	0.738464	0.0029
	78	53.3 ± 3.5 (10)	2.886008	8.680366	0.559166	<0.0001
	104	54.9 ± 4.0 (38)	3.781053	16.11593	0.550065	<0.0001
MCH (pg)	26	16.9 ± 0.2 (10)	–0.000000	1.009222	0.944239	0.5847
	52	16.5 ± 0.7 (10)	–2.019534	4.132121	0.749652	0.0039
	78	17.1 ± 0.9 (10)	2.741532	7.901004	0.596061	<0.0001
	104	17.6 ± 1.1 (38)	4.167626	20.93079	0.576434	<0.0001
MCHC (%)	26	34.3 ± 0.4 (10)	–0.810816	–0.947959	0.864727	0.0831
	52	32.1 ± 2.6 (10)	–2.947889	9.032577	0.543295	<0.0001
	78	32.1 ± 0.4 (9)	–0.232569	0.723986	0.970951	0.8978
	104	32.1 ± 0.5 (38)	–1.844773	5.161610	0.856344	<0.0001
Platelet ($\times 10^3$/mm^3)	26	600 ± 98 (10)	1.784901	3.528388	0.811905	0.0202
	52	625 ± 140 (10)	2.520460	6.950602	0.667335	0.0005
	78	628 ± 139 (10)	2.622228	7.649434	0.659365	0.0004
	104	640 ± 135 (38)	1.875646	8.745862	0.828173	<0.0001
White blood cell ($\times 10^3$/mm^3)	26	5.64 ± 0.73 (10)	0.672271	0.233766	0.955789	0.7228
	52	5.25 ± 1.30 (10)	1.045335	1.050182	0.895080	0.1843
	78	5.17 ± 1.96 (10)	1.185022	1.684577	0.913693	0.2942
	104	5.89 ± 1.82 (37)	1.896039	5.735049	0.854604	<0.0001
Protein (g/dL)	26	6.26 ± 0.13 (10)	1.899329	5.018747	0.808643	0.0185
	52	6.17 ± 0.17 (10)	–1.049502	1.758844	0.925659	0.3912
	78	6.29 ± 0.13 (10)	0.562836	0.959196	0.961841	0.7945
	104	6.33 ± 0.40 (10)	2.114829	5.444267	0.773978	0.0074

Table 2. Continued.

Measured items	Week after dosing	Mean ± S.D. (N)	Skewness	Kurtosis	*W* value	*P* (Prob<W)
Albumin (g/dL)	26	3.48 ± 0.04 (10)	–0.162619	–0.581849	0.990918	0.9972
	52	3.32 ± 0.15 (10)	–2.331799	6.208979	0.727428	0.0022
	78	3.31 ± 0.12 (10)	–0.704849	–0.438294	0.908930	0.2616
	104	3.03 ± 0.19 (10)	0.131861	–0.217544	0.984835	0.9836
Glucose (mg/dL)	26	148 ± 10 (10)	0.660299	1.159669	0.933194	0.4638
	52	141 ± 20 (10)	0.049999	0.035462	0.991977	0.9982
	78	136 ± 13 (10)	–0.843247	–0.447104	0.904604	0.2348
	104	121 ± 21 (10)	–1.918265	4.191633	0.789032	0.0110
Triglyceride (mg/dL)	26	41.6 ± 10.4 (10)	0.190987	–0.512488	0.956822	0.7352
	52	82.1 ± 8.8 (10)	0.469701	–1.092697	0.934169	0.4738
	78	55.9 ± 15.3 (10)	0.155934	–1.163010	0.951712	0.6736
	104	72.9 ± 79.4 (10)	2.863948	8.582074	0.572824	<0.0001
T. cholesterol (mg/dL)	26	56.2 ± 4.8 (10)	–0.382282	–0.048644	0.974070	0.9191
	52	75.2 ± 7.1 (10)	1.032991	0.547890	0.909721	0.2668
	78	88.9 ± 17.1 (10)	0.258985	–0.192289	0.964639	0.8261
	104	182 ± 163 (10)	2.942835	8.940449	0.535933	<0.0001
F. cholesterol (mg/dL)	26	13.9 ± 2.0 (10)	–0.762307	0.344792	0.900935	0.2140
	52	19.9 ± 2.3 (10)	1.531800	1.648699	0.789195	0.0110
	78	23.5 ± 5.1 (9)	0.171044	0.274932	0.986117	0.9877
	104	47.9 ± 39.9 (10)	2.743865	7.924692	0.600471	<0.0001
NEFA (μEq/L)	26	531 ± 51 (10)	–0.186268	–0.202387	0.966323	0.8445
	52	499 ± 72 (10)	–0.384472	–1.798228	0.847110	0.0519
	78	616 ± 106 (10)	–0.438186	–0.402537	0.942412	0.5637
	104	649 ± 192 (10)	1.717350	3.217589	0.830044	0.0328
Phospholipid (mg/dL)	26	105.6 ± 5.8 (10)	–0.642605	–0.291962	0.939032	0.5258
	52	128.8 ± 9.9 (10)	0.512305	–0.951732	0.909956	0.2684
	78	140.6 ± 19.7 (10)	0.747156	0.654728	0.958073	0.7502
	104	242.1 ± 198.4 (10)	2.986102	9.157032	0.519354	<0.0001
BUN (mg/dL)	26	19.2 ± 1.4 (10)	–0.140574	–1.465478	0.932193	0.4536
	52	19.1 ± 1.2 (10)	1.101829	1.726886	0.929657	0.4286
	78	18.3 ± 1.4 (10)	–1.210249	0.509227	0.845799	0.0501
	104	15.7 ± 1.7 (10)	–0.130295	–0.686202	0.960532	0.7793
Creatinine (mg/dL)	26	0.268 ± 0.02 (10)	–0.097275	–0.514268	0.930708	0.4388
	52	0.258 ± 0.02 (10)	0.140528	–0.889603	0.926361	0.3976
	78	0.316 ± 0.02 (10)	1.146009	1.262381	0.900130	0.2097
	104	0.274 ± 0.02 (10)	1.005894	1.387836	0.904489	0.2341
T. bilirubin (mg/dL)	26	0.02 ± 0.008 (10)	–0.000000	–1.392857	0.837167	0.0397
	52	0.040 ± 0.009 (10)	0.601381	0.396220	0.884298	0.1393
	78	0.038 ± 0.011 (10)	–0.660623	–0.708977	0.851929	0.0590
	104	0.060 ± 0.043 (10)	1.815353	5.214696	0.785586	0.0100
AST (U/L)	26	176 ± 55 (10)	0.902169	–0.554522	0.865010	0.0937
	52	220 ± 96 (10)	1.622326	2.650508	0.827957	0.0310
	78	319 ± 384 (10)	3.027770	9.374563	0.504224	<0.0001
	104	97 ± 39 (10)	1.078398	3.285330	0.890480	0.1637
ALT (U/L)	26	95 ± 33 (10)	1.454087	1.244859	0.784016	0.0096
	52	127 ± 45 (10)	1.519397	2.112544	0.836182	0.0387
	78	111 ± 38 (10)	0.344387	–0.899574	0.930464	0.4364
	104	54 ± 19 (10)	2.279570	5.923983	0.734225	0.0026

Data showing non-normal distribution in toxicity studies.

Table 2. Continued.

Measured items	Week after dosing	Mean ± S.D. (N)	Skewness	Kurtosis	*W* value	*P* (Prob<W)
ALP (U/L)	26	434 ± 34 (10)	–0.548534	–0.083400	0.957106	0.7386
	52	590 ± 195 (10)	1.888497	3.158020	0.730174	0.0024
	78	480 ± 51 (10)	0.068486	–1.162209	0.962040	0.7968
	104	423 ± 217 (10)	2.149472	6.596734	0.687817	0.0008
Gamma-GTP (U/L)	26	0.57 ± 0.13 (10)	0.334360	–0.851654	0.934464	0.4768
	52	2.99 ± 2.61 (10)	2.288782	5.874883	0.734093	0.0026
	78	3.02 ± 0.99 (10)	2.544844	7.340248	0.680303	0.0007
	104	4.53 ± 5.94 (10)	3.064098	9.546018	0.485653	<0.0001
CPK (U/L)	26	132 ± 71 (10)	1.405197	1.459845	0.826251	0.0297
	52	135 ± 54 (10)	2.028677	4.862049	0.783761	0.0096
	78	137 ± 51 (10)	0.679117	0.358963	0.956786	0.7348
	104	137 ± 49 (10)	0.679117	0.358963	0.956786	0.7348
Calcium (mg/dL)	26	9.97 ± 0.12 (10)	–1.028796	2.355838	0.908560	0.2592
	52	10.25 ± 0.26 (10)	0.622789	–1.215305	0.894208	0.1802
	78	10.03 ± 0.20 (10)	–0.447473	–0.227102	0.948258	0.6321
	104	10.25 ± 0.41 (10)	0.282217	0.683732	0.959512	0.7673
I. phosphourus (mg/dL)	26	5.24 ± 0.58 (10)	1.453477	2.898652	0.890158	0.1623
	52	5.25 ± 0.60 (10)	–0.265652	–1.319467	0.917625	0.3236
	78	5.16 ± 1.00 (10)	2.112960	5.009497	0.755524	0.0046
	104	4.37 ± 0.42 (10)	–0.225272	–1.069626	0.955620	0.7208
Sodium (mmol/L)	26	142.8 ± 0.7 (10)	–0.246562	–1.059510	0.952107	0.6786
	52	142.9 ± 1.2 (10)	0.154641	–1.784248	0.904093	0.2318
	78	142.6 ± 0.9 (10)	0.077439	–1.701820	0.914934	0.3032
	104	142.2 ± 0.9 (10)	0.678063	2.573093	0.890711	0.1646
Potassium (mmol/L)	26	4.33 ± 0.20 (10)	–1.287061	3.459417	0.840438	0.0434
	52	5.17 ± 1.74 (10)	2.795551	8.170148	0.581036	<0.0001
	78	4.67 ± 0.43 (10)	1.609681	4.564085	0.828575	0.0316
	104	4.60 ± 0.63 (10)	0.224616	3.836489	0.817432	0.0234
Chloride (mmol/L)	26	106.6 ± 0.9 (10)	0.132517	–1.296404	0.934956	0.4820
	52	105.9 ± 0.6 (10)	–0.693767	1.131234	0.917133	0.3198
	78	106.7 ± 0.6 (10)	–0.176520	1.076115	0.965682	0.8376
	104	107.5 ± 3.0 (10)	–2.615111	7.641016	0.963516	0.0002
Urine volume (mL/day)	26	8.9 ± 0.8 (10)	0.223450	–1.733729	0.811404	0.0199
	52	10.7 ± 1.1 (10)	0.727015	0.511967	0.915102	0.3045
	78	11.7 ± 2.2 (10)	0.165950	–0.297297	0.949578	0.6479
	104	15.3 ± 4.3 (10)	1.200014	2.100373	0.904073	0.2317
Specific gravity of urine	26	1.0714 ± 0.010(10)	–0.926281	0.461571	0.929365	0.4258
	52	1.0657 ± 0.004 (10)	–1.075203	0.613574	0.797283	0.0137
	78	1.0635 ± 0.008 (10)	–1.163222	1.543587	0.878260	0.1189
	104	1.0531 ± 0.015 (10)	–0.126186	–1.063711	0.955900	0.7204
Brain weight (g)	26	2.07 ± 0.03 (10)	0.107142	–0.007937	0.978767	0.9539
	52	2.13 ± 0.03 (10)	–1.189928	3.322323	0.881077	0.1280
	78	2.15 ± 0.04 (10)	0.230012	–1.155441	0.963657	0.8152
	104	2.18 ± 0.05 (38)	–0.211258	0.164836	0.979277	0.7763
Kidney weights (g)	26	2.07 ± 0.13 (10)	0.182205	–1.528715	0.919964	0.3422
	52	2.31 ± 0.13 (10)	–0.475888	–0.248882	0.964817	0.8281
	78	2.44 ± 0.12 (10)	–1.419510	1.990706	0.848660	0.0541
	104	2.58 ± 0.16 (38)	0.855444	1.129690	0.953775	0.1626

Table 2. Continued.

Measured items	Week after dosing	Mean ± S.D. (N)	Skewness	Kurtosis	*W* value	*P* (Prob<W)
Testes weights (g)	26	3.12 ± 0.08 (10)	–0.525184	–0.971205	0.934654	0.4788
	52	3.12 ± 0.14 (10)	–0.468497	–0.131631	0.896138	0.1894
	78	2.87 ± 0.21 (10)	0.800023	0.005163	0.934882	0.4812
	104	3.46 ± 1.14 (38)	–0.040877	–1.041625	0.961435	0.2827
Heart weight (g)	26	0.90 ± 0.04 (10)	0.179275	–1.220476	0.945784	0.6028
	52	1.02 ± 0.05 (10)	0.314630	–0.489476	0.962218	0.7988
	78	1.06 ± 0.05 (10)	–0.110908	–0.632629	0.953761	0.6983
	104	1.08 ± 0.08 (38)	1.227896	2.488375	0.916636	0.0087
Spleen weight (g)	26	0.559 ± 0.023 (10)	–0.047375	–0.906373	0.949593	0.6480
	52	0.734 ± 0.403 (10)	3.136537	9.880044	0.422118	<0.0001
	78	0.659 ± 0.121 (10)	2.690643	7.994618	0.634224	0.0002
	104	0.090 ± 1.092 (38)	5.753439	34.25695	0.291994	0.0000
Liver weight (g)	26	7.25 ± 0.46 (10)	0.479709	–0.312925	0.914679	0.3013
	52	8.24 ± 0.60 (10)	0.474113	–0.339653	0.970241	0.8845
	78	8.69 ± 0.48 (10)	–0.388715	–1.749992	0.869745	0.0949
	104	8.90 ± 1.17 (38)	1.479624	4.777876	0.904438	0.0033
Adrenal gland weights (mg)	26	41.1 ± 3.8 (10)	–0.050780	–2.086745	0.861759	0.0768
	52	43.0 ± 1.8 (10)	0.000000	–0.450000	0.984549	0.9826
	78	42.6 ± 2.7 (10)	0.895902	–0.265955	0.873469	0.1048
	104	54.6 ± 8.4 (38)	1.099387	1.315698	0.923173	0.0146
Brain weight/body weight (%)	26	0.284 ± 0.005 (10)	–0.490178	–1.139231	0.956116	0.7267
	52	0.609 ± 0.032 (10)	0.350032	–0.328267	0.954739	0.7101
	78	0.605 ± 0.018 (10)	0.208799	–1.568370	0.919411	0.3378
	104	0.607 ± 0.044 (38)	0.920363	0.669970	0.925392	0.0175
Kidney weights/body weight (%)	26	0.650 ± 0.031 (10)	0.002528	0.016579	0.990174	0.9963
	52	0.658 ± 0.034 (10)	0.664516	–0.368378	0.948706	0.6374
	78	0.687 ± 0.031 (10)	0.020987	–0.088839	0.984817	0.9835
	104	0.712 ± 0.041 (38)	–0.066129	–0.888781	0.962980	0.3143
Testes weights/body weight (%)	26	0.978 ± 0.041 (10)	–0.438854	–0.729543	0.931480	0.4465
	52	0.888 ± 0.050 (10)	0.603730	0.252793	0.958822	0.7591
	78	0.808 ± 0.055 (10)	0.262714	–1.691119	0.880900	0.1275
	104	0.971 ± 0.348 (38)	0.153011	–0.663607	0.967328	0.4175
Heart weight/body weight (%)	26	0.649 ± 0.031 (10)	–0.357780	–0.468103	0.921177	0.3522
	52	0.292 ± 0.019 (10)	1.709220	4.833894	0.811629	0.0201
	78	0.301 ± 0.016 (10)	0.871315	0.564719	0.930353	0.4354
	104	0.300 ± 0.023 (38)	2.249169	7.986800	0.831950	<0.0001
Spleen weight/body weight (%)	26	0.175 ± 0.005 (10)	–0.265826	0.336892	0.968468	0.8669
	52	0.211 ± 0.128 (10)	3.137660	9.885458	0.420608	<0.0001
	78	0.186 ± 0.038 (10)	2.819508	8.424454	0.589203	<0.0001
	104	0.283 ± 0.371 (38)	5.964813	36.22274	0.252599	<0.0001
Liver weight/body weight (%)	26	2.26 ± 0.08 (10)	–0.060752	–0.520184	0.967285	0.8547
	52	2.33 ± 0.09 (10)	–0.037230	–0.734432	0.974986	0.9265
	78	2.44 ± 0.09 (10)	–0.433644	0.000484	0.971505	0.8965
	104	2.46 ± 0.28 (38)	2.200957	6.845344	0.797850	<0.0001
Adrenal gland weights/body weight (%)	26	0.012 ± 0.001 (10)	0.478382	0.552276	0.931284	0.4445
	52	0.012 ± 0.000 (10)	0.806457	1.236994	0.836874	0.0394
	78	0.012 ± 0.001 (10)	0.994368	1.185267	0.840083	0.0430
	104	0.017 ± 0.016 (38)	5.928476	35.96717	0.281159	<0.0001

bution. According to Finney (1995) 'Bartlett test is notorious for its unwanted sensitivity to non-normality of error distribution, and is an untrustworthy instrument for classifying some data sets as homogeneous in variance, others as heterogeneous'. Data derived from toxicity studies fall into 3 categories-1. Normal distribution with homogenous of variation (Fig. 1), 2. Non-normal distribution with homogeneous of variance (Fig. 2) and 3. Non-normal distribution with heterogeneous of variance (Fig. 3). The present study clearly indicates that though the measured items in toxicity studies show a homogeneity in variance as per

Table 3. Distribution of measured items that did not show normality.

Distribution		Skewness	
		To right distribution (+)	To left distribution (–)
Kurtosis	Sharply (+)	MCV, MCH, Platelet, Protein, AST, ALT, ALP, Gamma-GTP, CPK, Free cholesterol, Potassium, Spleen weight, Heart, Spleen and adrenal gland weights/body weight	Food consumption, Hematocrit, Hemoglobin, Red blood cell, MCHC
	Uniformly (–)	None	None

Table 4. Number of measured items that showed a non-normal distribution.

Measured Items	Number of non-normal items on each week after dosing			
	Week 26	Week 52	Week 78	Week 104
Hematology	2	7	6	8
Biochemistry	4	8	3	12
Urinalysis	1	1	0	0
Organ weight	0	1	0	4
Organ weight/BW	0	2	1	5

Fig. 1. Normal distribution in two groups.

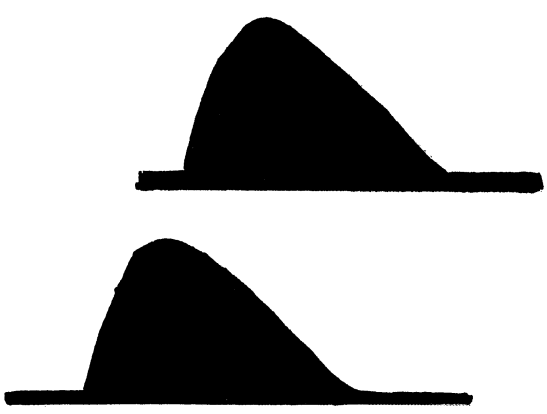


Fig. 2. Non-normal distribution and homogeneous of variance.

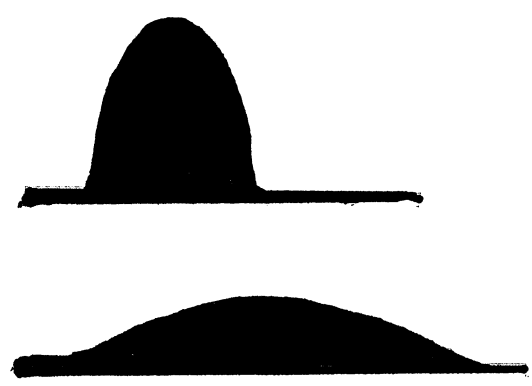


Fig. 3. Non-normal distribution and heterogeneous of variance.

Bartlett's test, many of them do not follow a normal distribution.

I suggest that selection of a statistical tool for analyzing measured items obtained from toxicity studies must be done after giving due consideration of 1. homogeneity of variance of data and 2. normal distribution of data. Normal distribution of the data may be assessed in the concurrent control group of toxicity studies and not from the historical data. If the data of the concurrent control group show a non-normal distribution, then an appropriate non-parametric test with robustness may be chosen to analyze these data.

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