Journal of Drug Metabolism and Toxicology

Saganuwan, J Drug Metab Toxicol 2015, 6:3
DOI: 10.4172/2157-7609.1000180

Research Article Open Access

Arithmetic-Geometric-Harmonic (AGH) Method of Rough Estimation of Median Lethal Dose (Ld50) Using Up – and – Down Procedure

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Received date: April 6,2015; **Accepted date:** April 29,2015; **Published date:** May 6,2015

Abstract

Earlier methods adopted for the estimation of median lethal dose ($LD_{50)}$ used many animals (40 – 100). But for the up – and – down procedure, 5 – 15 animals can be used, the number I still consider high. So this paper seeks to adopt arithmetic, geometric and harmonic (AGH) mean for rough estimation of median lethal dose (LD_{50}) using up – and – down procedure by using 2 – 6 animals that may likely give 1 – 3 reversals. The administrated doses should be summed up and the mean, standard deviation (STD) and standard error of mean (SEM) should be determined. The mean would be the LD_{50}, whereas standard deviation would be the default dose progression and standard error of mean would provide the lower and upper boundary of the LD_{50}, which may also serve as confidence interval. Arithmetic, geometric and harmonic means all go together harmoniously. If there is no death above 15,000 mg/kg body weight, the test agent is practically safe and that serves as the limit boundary for safety. The revised method saves time, reserves large numbers of animals that may be wasted and ignores complex mathematical manipulation involved and therefore encouraging the principles refinement, reduction and replacement (3R).

Keywords: Median lethal dose; up-and-down-procedure; Arithmetic mean; Standard deviation; Default dose; Standard error of mean

Introduction

Median lethal dose (LD_{50}) is the amount of test agent that can cause death in 50% of test animals. The LD_{50} concept was first introduced in 1927 by Trevan [1]. There are different methods used to estimate median lethal dose [2-10]. Up – and – down procedure was adopted several times [11-17]. The method involves the use of less number of animals (5 – 15), is faster and its findings are relatively comparable to the findings from other methods [18]. For all the methods, the study outcome is likely to be influenced by the choice of starting dose level(s), relative to the true LD_{50} value, especially in the case of shallow slopes [19]. Therefore, information on toxic effects seen only at dose levels close to a lethal dose will not be obtained [20]. Upon all the benefits being derived from the most recent and the past revised up – and – down procedure, there is hurdle of mathematical complexity involved using up – and – down procedure. Such mathematical complexity involves rough estimation of LD_{50} using geometric mean, maximum likelihood estimator, most confidence interval and a point estimate of the $LD_{50,}$ which is a high descriptor of toxicity of a chemical to a population. In order to remove all the hurdles mentioned above and decrease the number of test animals, I hereby present a simplest arithmetic method for rough estimation of LD_{50} using up – and – down procedure.

Revision of up – and – down procedure

Stitzel gave a hypothetical example [21-23] of how to estimate LD_{50} in "Test Guide Lines 425", using up – and – Down Procedure as follows:

Number	Dose (mg/kg)	Survival Status
1st	200	0
2nd	260	X
3rd	200	X
4th	154	0
5th	200	0
6th	260	X
Arithmetic Mean	212.3	50% (LD50)
Standard Deviation (SD)	41	1.6 (Default dose)

Citation: Saganuwan SA, (2015) Arithmetic-Geometric-Harmonic (AGH) Method of Rough Estimation of Median Lethal Dose (Ld50) Using Up – and – Down Procedure. J Drug Metab Toxicol 6: 180. doi:10.4172/2157-7609.1000180

Standard error of Mean (SEM)	16.7	7.9% (Confidence interval)
Geometric Mean	209	50% (LD50)
Harmonic Mean	212.3	50% (LD50)
Standard Deviation (SD)	41	1.6 (Default dose)
Standard Error of Mean	16.7	7.9% (Confidence interval)
Keys: X=Death, O Survival		

Table 1: Stitzel hypothetical example

Since there are three reversals, Stitzel calculated the geometric mean of all the doses and arrived at 209 mg/kg body weight [21]. My views about Stitzel estimation are: Arithmetic or harmonic mean can be used in place of geometric mean and that would give us 212.3 mg/kg body weight; Standard deviation (SD) of the either mean should serve as default dose progression which is logarithm of 41 mg/kg (1.6); and Standard error of the either mean (SEM) should also serve as lower and upper boundary of the LD_{50}. i.e. +16.7mg/kg realizing the fact that LD_{50} is not fixed. So our LD_{50} from Stitzel example should be 212+16.7 mg/kg body weight. Hence the LD_{50} estimated by Stitzel [21] is within the range of my LD_{50}. Saganuwan et al. estimated LD_{50} of aqueous extract of Abrus precatorius leaf using geometric mean10 as presented below:

No.	Dose (mg/kg)	Survival Status
1st	2559.5	X
2nd	974.5	O
3rd	2559.5	O
4th	4144.5	X
5th	2559.5	0
6th	4144.5	X
Arithmetic Mean	2823.7	50% (LD50)
Standard Deviation (SD)	1193.1	3.1 (Default dose)
Standard error of Mean (SEM)	487.2	17.2% (Confidence interval)
Geometric Mean	2558.8	50% (LD50)
Harmonic Mean	2823.7	50% (LD50)
Standard Deviation (SD)	1193.1	3.1% (Default dose)
Standard Error of Mean	487.2	17.2% (Confidence interval)

Table 2: Toxicity pattern of *Abrus precarious* leaf extract

The Geometric mean of the three reversals seen in Table 2 is 2558.8 mg/kg body weight. My views about Saganuwan et al. estimation [10] are: Arithmetic or harmonic mean can be used instead of geometric mean and that would give us 2823.7 mg/kg body weight; Standard deviation of the either mean should serve as default dose progression which is logarithm of 1193.1 mg/kg (3.1); Standard error of mean (SEM) should serve as lower and upper boundary of the LD50. i.e. + 487.2 mg/kg body weight, realizing the fact that LD_{50} is not fixed. Therefore, the LD_{50} (2823.7 + 487.2 mg/kg) reported by Saganuwan et al [10] should be between 2336.5 and 3310.9 mg/kg body weight. Hence, the LD_{50} of Saganuwan et al. falls within the range of my LD_{50}.

Discussion

The LD_{50} (209 mg/kg) estimated by Stitzel [23] is within the range of my estimated LD_{50} (205 to 229.0 mg/kg) signifying that arithmetic mean of three reversals can be used as rough estimate of LD_{50} and standard deviation can be used as default dose progression, whereas standard error of mean can serve as lower and upper boundary of the mean which may in turn represent confidence interval. But the LD_{50} (2558.8 mg/kg) estimated from Saganuwan et al. [10] is within the estimated range of my LD_{50} (2336.5 to 3310.9 mg/kg body weight). Saganuwan et al. [10, 22] and Saganuwan [9, 18] had earlier reported that median lethal dose of aqueous extract of Abrus precatorius leaf is between 2559.5 and 3011.4 mg/kg body weight. Therefore, there is precision, validity and reliability of using arithmetic mean as rough estimate of LD_{50}, standard deviation as default dose progression and standard error of mean as lower and upper boundary. The arithmetic, geometric and harmonic means are Pythagorean means. In their calculating, the arithmetic mean is greater than or equal to the geometric mean, which is greater than or equal to the harmonic mean. The geometric mean is particularly appropriate for exponential type of data. The harmonic mean is good for things like rates and ratios where an arithmetic mean would actually be incorrect. But when the sample size is unequal, the far and away most common procedure uses the harmonic mean of sample sizes. As a result, an unbalanced design will have less statistical power because the average samplesize will tend toward the least sample. The mean uses every value in the data and hence is a good representative of the data. Repeated samples drawn from the sample population tend to have similar means. The mean is therefore the measure of central tendency that best resists the fluctuation between different samples [25]. It is closely related to standard deviations, the most common measure of dispersion [24]. The important disadvantage of mean is that it is sensitive to extreme values/outliers, especially when the sample size is small [26]. Therefore, it is not an appropriate measure of central tendency for skewed distribution [27]. Harmonic mean is appropriate in situations where the reciprocals of values are more useful. It is used for determination of the average sample size of a number of groups, each of which has a different sample size. Geometric mean is an appropriate measure when values change exponentially and in case of skewed distribution that can be made symmetrical by a log transformation. It cannot be used if any of the values are zero or negative [24]. However, because small numbers of animals were used, the actual level of confidence was generally not exact [22]. The random stopping rule in UDP improves the ability of the test overall to respond to varying underlying conditions, but also causes the reported level of confidence and the actual level of confidence to differ somewhat [23]. Of all the principles of the three alternatives (fixed dose; acute toxic class and up – and - down), up – and – down, is the most suitable, because it gives endpoint – evident toxicity and obeys stopping criteria to limit number of animals used, using only 2 – 6 animals with possible 1 – 3 deaths [24]. If there is no death above 15,000 mg/kg body weight, the test agent is practically safe and that serves as the limit boundary for safety [28-30]. The revised method saves time, reserves large numbers of animals that may be waisted and ignores complex mathematical manipulation involved and so encouraging the principles of refinement, reduction and replacement (3R).

In conclusion, either arithmetic or harmonic mean can be used in place of geometric mean as rough estimate of median lethal dose (LD50) using up-and-down procedure since arithmetic, geometric and harmonic means go together harmoniously.

References

1. Trevan JW (1927) The error of determination of toxicity. Proceeding of the Royal Society (London) Series B 101 483-514.
2. Karber G (1931) Reihenversucbe. Arch Exper Pathol Pharmacol 162: 480-483.
3. ASTM (1987) Standard Test Method for Estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials, Philadelphia, USA.
4. Dixon WJ (1991) Staircase bioassay: the up-and-down method. Neurosci Biobehav Rev 15: 47-50.
5. Biacchesi S, Skiadopoulos MH, Yang L, Murphy BR, Collins PL, et al. (2005) Rapid human metapneumovirus microneutralization assay based on green fluorescent protein expression. J Virol Methods 128: 192-197.
6. Miller LC, Tainter ML (1944) Examination of LD50 and ED50 values and errors using logarithm probity paper. Proc Soc Exper Biol Med 57: 261-264.
7. litchfield JT Jr, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96: 99-113.
8. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 53: 275-289.
9. Saganuwan SA (2011) A modified arithmetical method of Reed and Munch for determination of a relatively ideal median lethal dose (LD50). Afr J Pharm Pharmacol 5: 1543-1546.
10. Saganuwan SA, Onyeyili PA, Suleiman AO (2011) Comparative toxicological effects of orally and intraperitoneally administered aqueous extract of Abrus precatorius leaf in Mus musculus. Herba polonica 57: 34-44.
11. OECD (1987) Guideline for the Testing of Chemicals. Testing Guideline 401, Acute Oral Toxicity. OECD Paris.
12. OECD (2000) Guideline Document on Acute Oral Toxicity Testing. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24.
13. OECD (2000) Guideline Document on the Recognition Assessment and Use of Clinical Signs as Human Endpoints for Experimental Animals used in Safety Evaluation. Environmental Health and Safety Monograph Series on Testing and Assessment No. 19.
14. OECD (2001a) Test Guideline 420. Acute Oral Toxicity â€" Fixed Dose Procedure.
15. OECD (2001b) Test Guideline 423. Acute Oral Toxicity â€" Acute Toxic Class Method.
16. OECD (2001c) Test Guideline 425. Acute Oral Toxicity â€" Up â€" and â €" Down Procedure.
17. United Nations Economic Commissions for Europe (UN/ECE) (2003): Globally Harmonized System of Classification and Labeling of Chemicals (6HS). UN, New York and Geneva.
18. Saganuwan SA (2012) Principles of Pharmacological calculations (1st edn) Ahmadu Bello University Press Zaria 529.
19. Gribaldo L, Gennari A, Blackburn K, Clemedson C, Deguercy A, et al. (2005) Acute toxicity. Altern Lab Anim 33 Suppl 1: 27-34.
20. van den Heuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJ, et al. (1990) The international validation of a fixed-dose procedure as an alternative to the classical LD50 test. Food Chem Toxicol 28: 469-482.
21. Stitzel K (2000) Overview Test guidelines 425, up â"and â" down Procedure. Up â "and â "Down Procedure Peers Panel Report, the Procters and Gamble Company, J15-17.
22. Jennison C, Turnbull BW (2000) Group Sequential Methods with Applications to clinical trials. Chapman and Hall, CRC Boca Raton, FL.
23. Botham PA (2004) Acute systemic toxicity--prospects for tiered testing strategies. Toxicol In Vitro 18: 227-230.
24. Manikandan S (2011) Measures of central tendency: The mean. J Pharmacol Pharmacother 2: 140-142.
25. Glaser AN (2000) High Yield Biostatistics (1st edn) Lipincott William and Wilkins, New Delhi, India.
26. Dawson B, Trapp RG (2004) Basic and Clinical Biostatistical (4th edn) Mc-Graw Hill, New York, USA.
27. Swinscow TD, Campbell MJ (2003) Statistics at Square One (10th edn) Viva Books Private Ltd, New Delhi, India.
28. Shiryaev AN, Spokoing VG (2000) Statistical Experiments and Decisions. Statistical Inference for Autoregressive Models of the First Order Asymptomtic Theory. World Scientific Publication, London, Singapore.
29. Hodge HC, Sterner JH (1949) Tabulation of toxicity classes. See comment in PubMed Commons below Am Ind Hyg Assoc Q 10: 93-96.
30. Gosselin RE, Smith SP, Hodge HC (1984) Clinical Toxicology of Commercial Products, Williams and Wilkins, London, UK.