Journal of Environmental Biology

January 2011, **32 (1)** 11-16 (2011)

Statistical tools for analysing the data obtained from repeated dose toxicity studies with rodents: A comparison of the statistical tools used in Japan with that of used in other countries

Author Details

Katsumi Kobayashi (*Corresponding author*)	National Institute of Technology and Evaluation (NITE), 2-49-10 Nishihara, Shibuya-ku, Tokyo - 151 0066, Japan e-mail: kobayashi@beige.plala.or.jp
K. Sadasivan Pillai	Frontier Lifeline, International Centre for Cardio-Thoracic and Vascular Diseases, Chennai - 600 101, India
Soma Guhatakurta	Frontier Lifeline, International Centre for Cardio-Thoracic and Vascular Diseases, Chennai - 600 101, India
K.M. Cherian	Frontier Lifeline, International Centre for Cardio-Thoracic and Vascular Diseases, Chennai - 600 101, India
Mariko Ohnishi	National Institute of Technology and Evaluation (NITE), 2-49-10 Nishihara, Shibuya-ku, Tokyo 151-0066, Japan

Abstract

In the present study, an attempt was made to compare the statistical tools used for analysing the data of repeated dose toxicity studies with rodents conducted in 45 countries, with that of Japan. The study revealed that there was no congruence among the countries in the use of statistical tools for analysing the data obtained from the above studies. For example, to analyse the data obtained from repeated dose toxicity studies with rodents, Scheffé's multiple range and Dunnett type (joint type Dunnett) tests are commonly used in Japan, but in other countries use of these statistical tools is not so common. However, statistical techniques used for testing the above data for homogeneity of variance and inter-group comparisons do not differ much between Japan and other countries. In Japan, the data are generally not tested for normality and the same is true with the most of the countries investigated. In the present investigation, out of 127 studies examined, data of only 6 studies were analysed for both homogeneity of variance and normal distribution. For examining homogeneity of variance, we propose Levene's test, since the commonly used Bartlett's test may show heterogeneity in variance in all the groups, if a slight heterogeneity in variance is seen any one of the groups. We suggest the data may be examined for both homogeneity of variance and normal distribution. For the data of the groups that do not show heterogeneity of variance, to find the significant difference among the groups, we recommend Dunnett's test, and for those show heterogeneity of variance, we recommend Steel's test.

Publication Data

Paper received:
01 December 2009

Revised received:
April 24 2010

Accepted:
11 May 2010

Key words

Statistical tools, Toxicity study, Rodents, Cluster analysis, Dunnett's test, Steel's test

Introduction

It is a regulatory requirement in most of the countries that toxicity studies are conducted in animal models as per appropriate guidelines for registering industrial chemicals with the respective Government agencies of the countries. Though most of the regulatory guidelines, for example, OECD (1995), EPA (2000) and FDA (2003) give sufficient information on the conduct of repeated dose toxicity studies with rodents, none of them gives a clear picture on the statistical tools to be used for analysing the data obtained from these studies. However, it is mentioned in these guidelines that the statistical methods should be selected during the design of the study. Selection of a non-appropriate statistical tool during the design of the study or using a different statistical tool from that mentioned in the study plan with improper justification at the end of the study may lead to

Table - 1: Statistical methods used in various countries to analyse the data obtained from repeated dose toxicity studies with rodents

Country	[1]Statistical method, [2]Test substance, [3]Reference
Algeria	[1] Student *t*-test, [2] Ammonium nitrate, [3] *African J. Biotech*., **5**, 749-754, 2006.
Algeria	[1] (*t*) student test and Dunnett method, [2] Diflubenzuron, [3] *Sci. Res. Essay*, **2**, 79-83, 2007.
Argentina	[1] Kruskall-Wallis test, ANOVA, [2] 1, 2-dimethylhydrazine, [3] *Biocell* (Mendoza), **26,** 3, Mendoza ago./dez., 2002.
Argentina	[1] ANOVA, Student-Newman-Keuls test, [2] Chitosan, [3] *J. Leukocyte Biol*., **78**, July 2005.
Belgium	[1] ANOVA, Tukey honest test, Kolmogorov-Smirnov test [2] Bupivacaine and ropivacaine, [3] *Anesth. Analg*., **91**, 1489-1492, 2000.
Belgium	[1] ANOVA, [2] Chitosan-DNA nanoparticles**, [3]** *AAPS Pharm. Sci. Tech*., **5**, 2004.
Brazil	[1] ANOVA, [2] Methylmethacrylate**, [3]** *Braz. Oral Res*., **19** Sao Paulo, July/Sept., 2005.
Brazil	[1] ANOVA followed by the Tukey multiple comparison test, [2] *Cordia salicifolia* extract, [3] *Acta Sci. Health Sci*., **27**, 4144, 2005.
Cameroon	[1] ANOVA followed by the Student-Newman Keuls test, [2] Hydro-ethanolic extract of leaves of *Senna alata* (L.), [3] *African J. Biotech*., **5**, 283-289, 2006.
Cameroon	[1] ANOVA and Duncan's multiple range tests, [2] *Hibiscus cannabinus*, [3] *African J. Biotech*., **4**, 833-837, 2005.
Canada	[1] One-way ANOVA followed by Newman–Keuls test [2] Amylin Receptor Blocks-Amyloid, [3] *J. Neurosci*., **24**, 5579-5584, 2004.
Canada	[1] Student's *t*-test, ANOVA, Dunnett's multiple comparison test, [2] Hexachlorobenzene, [3] *Environ. Health Perspectives*, **111,** 4, 2003.
Chile+Sweden	[1] *F*-ANOVA and a *post-hoc* test (Fisher's protected partial least square test**)**, [2] Substantia Nigra and Neostriatum, [3] *J. Neurochem*.., **83**, 645–654, 2002.
Chile	[1] ANOVA followed by a *post hoc* Newman–Keuls' multiple comparison test**,** Student's *t* test, [2] Manganese, [3] *Pharm. Biochem*. Behavior, **77,** 245–251, 2004.
Chile, Costa Rica & Pararuay	[1] ANOVA followed by Dunnett's multiple comparison test, [2] *Aloysia polystachya*, [3] http://captura.uchile.cl/dspace/bitstream/2250/2329/1/Mora_S_Anxiolytic.pdf.
China	[1] One-way ANOVA, [2] Monosialoganglioside, [3] *Acta Pharmacol. Sin*, **25,** 727-732, 2004.
China	[1] Student's *t*-test, [2] Pyrethroid, [3] *J. Occup. Health*, **38**, 54-56, 1996.
China	[1] One-way ANOVA, Kruskal-Wallis test, Mann-Whitney *U* test, [2] **Hyperbaric oxygen preconditioning, [3]** *Chinese Med. J*., **113**, 837-839, 2000.
China	[1] One-way ANOVA, [2] GM1 ganglioside, [3] *J. Zhejiang Univ. Sci. B*., **6** (4), 254-258, 2005.
Cuba	[1] Normality assumptions (Kolmogorov-Smirnov's and Shapiro-Wilk's tests), Levene's test, ANOVA, Kruskall-Wallis's test, *t* paired tests or Wilcoxon's test, [2] Granulocyte-Colony Stimulating Factor (G-CSF), [3] *Biotecnología Aplicada*, **22,** 50-53, 2005.
Cuba	[1] Mann-Whitney test, [2] D-002, [3] *Biotecnología Aplicada*, **18**, 88-90, 2001.
Cuba	[1] ANOVA, Students–Newman–Keuls *post hoc* test, [2] Kainate, [3] *Eur. J. Pharmacol*., **390**, 295-298, 2000.
Czech Republic	[1] *t*-test after *F*-test, Normal distribution, [2] Aflatoxin B1 and T-2 toxin, [3] *Vet. Med. Czech*, **46**, 301-307, 2001.
Czech Republic	[1] One-way ANOVA test, Tukey-Kramer's *post hoc* test, [2] D-galactosamine, [3] *Physiol. Res*., **55,** 551-560, 2006.
Denmark	[1] Mann-Whitney U test and ANOVA, [2] Novispirin G10, [3] *Antimicrobial Agent and Chemotherapy*, **49**, 3868-3874, 2005.
Denmark	[1] One-way ANOVA, Student's paired *t* test**,** [2] Bendroflumethiazide, [3] *J. Pharmacol. Exp. Ther*., **299,** 307-313, 2001.
Denmark	[1] Shapiro Wilks test, Levene's test, General linear model (GLM) analysis, [2] Diesel exhaust particles, [3] *Carcinogenesis*. **24**. 1847-1852, 2003.
Egypt	[1] One way ANOVA followed by Tukey–Kramer test for multiple comparison, [2] Benzo(*a*)pyrene, *Nigella sativa* seeds, [3] *Food Chem*. Toxicol., **45**, 88-92, 2007.
Egypt	[1] Student's *t* test, [2] Garlic extract, [3] *Res. J. Med. Medical Sci*., **1**, 85-89, 2006.
Finland	[1] ANOVA followed by Student Newman-Keuls test, [2] Oxygen, [3] *Eur. Respir. J*., **9**, 2531-2536, 1996.
Finland	[1] Mann-Whitney nonparametric *U* test, Kruskall-Wallis analyses with a Dunn's post test, [2] Doxorubicin, [3] *Cancer Res*., **61**, 6423–6427, 2001.
France	[1] ANOVA, Student *t* test, [2] Amphotericin B, [3] *Antimicrob. Agents Chemother*., **35**, 1303–1308, 1991.
France	[1] Bartlett's test, ANOVA, Dunnett's test, Kruskal-Wallis test, Dunn's test, [2] 5á-reductase (5áR) inhibitor, [3] *Molecular & Cellular Proteomics*, July 12, 2006.
France	[1] ANOVA, Dunnett's test, [2] Ethylene oxide, [3] *Fund. Appl. Toxicol*., **34**, 223-227, 1996.
Germany	[1] ANOVA, Ryan-Einot-Gabriel-Walsh test, Nonparametric test (van der Waerden test using normalized scores), [2] Polybrominated diphenyl ethers, [3] *Environ. Health Perspectives*, **114**, 2006
Germany	[1] Two-factor analysis of variance with a Bonferroni correction, [2] Polychlorinated biphenyls (PCBs), [3] *Environ. Health Perspectives*, *109, 2001.*
Greece	[1] Two-way analysis of variance, LSD, [2] Urethan, [3] *J. Appl. Physiol*., **81,** 2304-2311, 1996.
Greece	[1] One-way analysis of variance and an unpaired Student's *t*-test, [2] BPV, [3] *Anesth. Analg*., **85,** 1337-1343, 1997.
Hungary	[1] *t* test, [2] 3-nitropropionic acid, [3] *Arh. Hig. Rada. Toksikol*., **56**, 297-302, 2005.
Hungary	[1] One-way ANOVA with LSD *post hoc* test, after the Kolmogorov-Smirnov normality, [2] Heavy metals, organophosphates, [3] *Arh. Hig. Rada.Toksikol*., **56**, 257-264, 2005.
India	[1] ANOVA and Dunnett test, Student *t* test, [2] Polyoxyethylene glycol, [3] *AAPS Pharm. Sci*., **6,** 2004.
India	[1] One-way ANOVA, Student's *t*-test, [2] Galactose, [3] *Human Reproduction*, **18**, 2031-2038, 2003
India	[1] Bartlett's test, ANOVA and Student's *t* test, [2] Fluoride, [3] *Fluoride*, **30**, Research Report 105, 1997.
India	[1] ANOVA and Dunnett test, Student *t* test, [2] Novel surfactants, [3] *AAPS Pharm. Sci*., **6**, Article 14, 2004.
India	[1] ANOVA, Tukey's test, [2] PUFA concentrate, [3] *African J. Biotech*, **6,** 1021-1027, 2007.
Iran	[1] ANOVA followed by multiple comparison test of Newman-Keuls test, [2] *Fumaria parviflora* Lam., [3] *DARU*, **12**, 136-140, 2004.

Iran	[1] Kruskal-Wallis test, [2] Valproic acid, [3] *DARU*, **14**, No. 1, 2006
Israel	[1] Shaphiro-Wilk, ANOVA, Tukey-Kramer, [2] Nitrogen and helium, [3] *J. Appl. Physiol*., **98,** 144-150, 2005.
Israel	[1] Student's *t* test, [2] AS 101, [3] *J. National Cancer Inst*., **88**, 1996.
Israel	[1] Wilcoxon rank-sum test, [2] *Juglans regia* L., *Olea europea* L., *Urtica dioica* L. and *Atriplex halimus* L., [3] eCAM Advance Access Published online on May 17, 2007.
Italy	[1] Student *t* test, [2] HD-Ad Vector, [3] *PNAS*, **102,** 3930-3935, 2005.
Italy	[2] ANOVA followed by Tukey test, [2] MDMA, [3] *BMC Neuroscience*, **7,** 13, 2006.
Japan	[1] Bartlett's test, Dunnett's test, Scheffe's test, Kruskal-Wallis ranking analysis, Dunnett type (Hollander and Wolf), [2] 4-nitrophenol and 2,4-dinitrophenol, [3] *J. Toxicol. Sci*., **26,** 299-311, 2001.
Japan	[1] Student *t*- test, [2] 5-FU, [3] *J. Toxicol. Sci*., **27,** 49-56, 2002.
Japan	[1] Bartlett's test, one-way layout analysis of variance, Kruskal-Wallis test, Mann-Whitney's *U* test, Dunnett type (Hollander and Wolf), [2] 3-Aminiphenol, [3] *Toxicol. Sci*., **27**, 411-421, 2002.
Japan	[1] Bartlett's test, Dunnett's or Scheffé's tests, Kruskal-Wallis ranking test, Dunnett type, Scheffe type or Mann-Whitney's *U* tests, [2] 3-methylphenol, [3] *J. Toxicol. Sci*., **28**, 59-70, 2003.
Japan	[1] Dunnett's multiple comparison test, [2] 1-carboxy-5,7- dibromo-6-hydroxy-2,3,4- trichloroxanthone, [3] *J. Toxicol. Sci*., **28,** 445-453, 2003.
Japan	[1] Bartlett test, Dunnett's test, Dunnett type rank sum test, [2] Wormwood, [3] *J. Toxicol. Sci*., **28,** 471-478, 2003.
Japan	[1] Dunnett's test, ANOVA, [2] Rice bran glycosphingolipid, [3] *J. Toxicol. Sci*., **29**, 73-80, 2004.
Japan	[1] Dunnett's multiple comparison test, [2] Dunnett's multiple comparison test, [3] *J. Toxicol. Sci*., **29,** 505-516, 2004.
Japan	[1] Bartlett's test, Dunnett's test, Steel's multiple comparison test, Dunnett type (Hollander and Wolf) Student's *t*-test, Aspin-Welch's *t* test, [2] 1, 3-dibromopropane, 1, 1, 2, 2-Tetrabromoethane, [3] *J. Toxicol. Sci.,* **30**, 29-42, 2005.
Japan	[1] ANOVA, Dunnett's or Scheffe's multiple comparison procedures, [2] 2,2'-isobutylidenebis (4,6-dimethylphenol), [3] *J. Toxicol. Sci*., **30**, 275-285, 2005.
Japan	[1] Dunnett' multiple comparison test, [2] 2,3,7,8-tetrabromodibenzo-*p*-dioxin, [3] *J. Toxicol. Sci*., **32**, 47-56, 2007.
Japan	[1] Bartlett's test, Dunnett's test, Dunnett-type method, [2] Water-Miscible Coenzyme Q10, [3] *J. Health Sci*., **51**, 346-356, 2005.
Japan	[1] Bartlett, ANOVA, Kruskal-Wallis, Dunnett, Scheffe, Dunnett type, Scheffe type, [2] 2,3,3,3,2',3',3',3'-Octachlorodipropyl ether (S-421), [3] *Bull. Natl. Inst. Health Sci*., **121**, 40-47, 2003.
Japan	[1] Dunnett's test or a Dunnett-type rank-sum test, [2] Bismuth, [3] *J. Occup. Health*, **47**, 293-298, 2005.
Japan	[1] Bartlett's test, one-way ANOVA, Dunnett Kruskal-Wallis rank sum test, Dunnett type, [2] p-dichlorobenzene (pDCB), [3] *J. Occup. Health*, **47**, 249-260, 2005.
Jordan	[1] ANOVA, Wilcoxon-rank sum test, [2] *Ferula harmonis* 'zallouh', [3] *Int. J. Impotence Res*., **13**, 247-251, 2001.
Jordan	[1] *t* test, [2] Plant Extract NASFIT, [3] *Int. J. Impotence Res*., **13**, 247-251, 2001.
Korea	[1] Levene's test, Dunnett's *t*-test, ANOVA, *t*-test, [2] Organic germanium fortified yeasts, [3] *J. Toxicol. Sci*., **29**, 541-553, 2004.
Korea	[1] ANOVA followed by a modified Z-test (LSD), [2] Nitric oxide, [3] *Invest. Ophthalmol. Visual Sci*., **38**, 995-1002, 1997.
Korea	[1] ANOVA by Bonferroni's *post hoc* comparison, [2] *Pinellia ternata* extract, [3] *Biol. Pharm. Bull*., **29**, 1278-1281, 2006.
Malaysia	[1] General Linear Model, one-way ANOVA, Scheffe test, Kruskal Wallis test, Mann-Whitney test, [2] Aqueous extract of *Labisia pumila var. alata* (LPA) or 'Kacip Fatimah', [3] *Indian J. Pharmacol*., **39**, 30-32, 2007.
Malaysia	[1] Student's *t*-test, [2] Yohimbine, [3] *Biokemistri*, **15,** 50-56, 2003.
Mexico	[1] Student's *t*, Mann-Whitney, [2] Follicle-Stimulating hormone, [3] *Environ. Health Perspectives*, **113**, 2005.
Mexico	[1] ANOVA followed by Fisher's least significant difference (LSD), [2] Perfluorooctane sulfonate, [3] *Environ. Health Perspectives*, 2003.
Mexico	[1] ANOVA, [2] Ethanol, [3] *Pharmacol*., **282**, 1028-1036, 1997.
Netherlands	[1] Dunnett's tests, [2] Butyl benzyl phthalate, [3] *Toxicol. Sci*., **94**, 282-292, 2006.
Netherlands	[1] Student's *t* test, [2] *N*-Acetylcysteine Protects, [3] *Am. J. Respir. Crit. Care Med*., **157**, 1283-1293, 1998.
Nigeria	[1] Student's *t*-test, [2] Ethanol extract of the leaves of *Datura stramonium*, [3] *African J. Biotech*., **6**, 1012-1015, 2007.
Nigeria	[1] Student's *t*-test, [2] *Trypanosoma brucei*-infected, [3] *African J. Biotech*., **5**, 1557-1561, 2006.
Nigeria	[1] ANOVA, Duncan's multiple range tests, [2] Lemongrass and green tea, [3] *African J. Biotech*., **5,** 1227-1232, 2006.
Nigeria	[1] ANOVA, Duncan's multiple range tests, [2] Crude oil, [3] *African J. Biotech*., **3**, 346-348, 2004.
Nigeria	[1] Student's *t*-test, [2] Salicylic acid and anthranilic acid, [3] *African J. Biotech*., **3**, 426-431, 2004.
Pakistan	[1] ANOVA and Dunnett's multiple test, [2] GM3 cancer vaccine, [3] *Pakistan J. Biol. Sci*., **87,** 1045-1050, 2005.
Pakistan	[1] Student's *t*-test, [2] Tanacetum, [3] *Arch. Pharm. Res*., **30**, 303-312, 2007.
Philippines	[1] Student *t*-test, ANOVA, [2] Ethylenebisdithiocarbamates, [3] *Environ. Health Perspectives*, 112, 2004.
Poland	[1] Student's one-tailed *t*-test, [2] Excitatory amino acid-induced toxicity, [3] *Acta Neurobiol. Exp*., **60**, 365-369, 2000.
Poland	[1] ANOVA followed by Student's *t*-test, Newman-Keuls test, Mann-Whitney ranking test, [2] (S)-3,5-DHPG, [3] *Pol. J. Pharmacol*., **54**, 11-18, 2002.
Poland	[1] Student's *t*-test, [2] Prednisolone, [3] *Pharm. Report*, **59**, 59-63, 2007.
Poland	[1] ANOVA, Newman-Keules test, [2] Endothelin-1, [3] *Pharma. Report*, **59**, 98-105, 2007.
Poland	[1] ANOVA followed by Duncan's test or Student's *t*-test, [2] Caffeine, [3] *Pharm. Report*, **59**, 296-305, 2007.
Poland	[1] One-way ANOVA followed by *post hoc* Duncan's test, Kruskal-Wallis ANOVA, Mann-Whitney *U*, [2] Methotrexate, [3] *Pharm. Report*, **59**, 359-364, 2007.
Portugal	[1] Paired *t* test, two-way ANOVA, Tukey's, [2] ETB receptor, [3] Society for Experimental Biology and Medicine, Department of Physiology, Faculty of Medicine, University of Porto, Portugal, 2006.

Portugal	[1] Two-Way ANOVA, followed by Bonferroni *pos hoc* test, [2] Amphetamine sulfate and HA bromide, [3] *J. Health Sci*., **53**, 371-377, 2007.
Republic of Slovenia	[1] Scheffe's-test**,** ANOVA, [2] 2,4-dichlorophenoxyacetic acid, [3] *Acta Vet. BRNO*, **68**, 281-229, 1999.
Russia	[1] Student's –test, [2] Olypiphate, [3] *Exp. Oncol*., **25**, 256-259, 2003.
Russia	[1] One-way ANOVA followed by Dunnett's test, [2] Methamphetamine, [3] *JPET*, **288**, 1298-1310, 1999.
Saudi Arabia	[1] Snedecor and Cochran, [2] *Euphorbia heliscopia*, [3] *Pakistan J. Nutr*., **5**, 135-140, 2006.
Saudi Arabia	[1] One-way ANOVA followed by Dunnet's or Tukey's for multiple comparison tests, [2] $HgCl_2$ & DMPS, [3] *Med. Sci. Monit*., **12**, 95-101, 2006.
Slovakia	[1] Unpaired Student's *t*-test and the Mann-Whitney *U*-test, [2] Pyridoindole antioxidant stobadine, [3] *Mol. Vision*, **11**, 56-65, 2005.
Slovakia	[1] Student's *t*-test, linear regression analysis with Pearson´s correlation coefficient, [2] Rooibos Tea *(Aspalathus linearis)*, [3] *Physiol. Res*., **53,** 515-521, 2004.
Slovakia	[1] Student's *t* test, [2] Liver preservation solution, [3] *Transplantation*, **70**, 430-436, 2000.
South Africa	[1] ANOVA, [2] Fumonisin B1, [3] *Carcinogenesis*, **25**, 1257-1264, 2004.
South Africa	[1] Student's *t*-test, [2] Ethanol and/ or chloroquine fed normal or low protein diet, [3] *Internet J. Hematol*., ISSN: 1540-2649, 1996 to 2008. http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijhe/vol3n1/chloroquine.xml
Spain	[1] Shapiro-Wilks test, Levene test, ANOVA, followed by Tukey, Kruskal-Wallis test, Mann-Whitney *U* test, [2] Ochratoxin A, [3] *Food Chem. Tox*., **42**, 825-834, 2004.
Spain	[1] ANOVA , [2] Pomegranate ellagitannin, [3] *J. Agric. Food Chem*., **51**, 3493-3501, 2003.
Spain	[1] 2-way ANOVA followed by the Bonferroni method for multiple comparisons, Kruskal-Wallis test and the Mann-Whitney *U*-test, [2] Uranyl acetate dehydrate, [3] *Exp. Biol. Med.,* **228**, 1072-1077, 2003.
Spain	[1] Student's t-test, [2] Monoamine, [3] *Neuropsychopharmacology*, **25**, 204-212, 2001.
Sweden and Netherlands	[1] ANOVA, Kruskal-Wallis test, Mann-Whitney *U* test and Dunnett, [2] PentaBDE, [3] *Organohalogen compounds*, **68**, 2006.
Sweden and Netherlands	[1] Two-way ANOVA, Student *t*-test, [2] Hexabromocyclododecane, [3] *Toxicol. Sci*., **94**, 281-292, 2006.
Switzerland	[1] Student's *t*-test, [2] Artemether, [3] *Am. J. Trop. Med. Hyg*., **66**, 30-34, 2002.
Switzerland	[1] ANOVA and Dunnett´s *post hoc* test, [2] Amiodarone and amiodarone derivatives, [3] JPET Fast Forward. Published in September 13, 2006
Thailand	[1] ANOVA, Duncan multiple range test, [2] Guttiferae, [3] http://www.grad.chula.ac.th/gradresearch6/pdf/96.pdf
Thailand	[1] One-way ANOVA, Dunnett multiple ranges test, [2] *Hyptis suaveolens*, [3] *Songklanakarin J. Sci. Technol*., **27**, 2005.
Thailand	[1] ANOVA, Duncan multiple range test, [2] Methomyl [3] *Arch. Hig. Rada. Toksikol*., **49**, 231-238, 1998.
Thailand	[1] ANOVA, [2] Topical Formulation of *Hyptis suaveolens* oil, [3] *CMU J*., **5**, 369-379, 2006.
Turkey	[1] Kruskal-Wallis, Dunn's multiple comparison tests, [2] Ofloxacin, [3] *Turk . J. Med. Sci*. **30**, 441-447, 2000.
Turkey	[1] ANOVA, [2] Vitamin C, [3] *Gen. Physiol. Biophys*., **24**, 47-55, 2005.
Turkey	[1] ANOVA, Tukey and Dunnett tests, [2] *Momordica charantia* L. (Bitter melon) fruit extract, [3] *African J. Biotech*., **6**, 273-277, 2007.
United Kingdom	[1] Student's *t*-test, [2] 3-(4-methylbenzylidine) camphor, [3] *Environ. Health Perspectives*., **110**, 2002.
United Kingdom	[1] ANOVA and analysis of covariance (ANCOVA), [2] Mixtures of estrogens, [3] *Environ. Health Perspectives*, **112**, 2004.
U.S.A.	[1] ANOVA, Bartlett's test, Dunnett test, [2] TiO_2 Rods and Dots, [3] *Toxicol. Sci*., **91**, 227-236, 2006.
U.S.A.	[1] ANOVA, Bartlett's test, Dunnett test, [2] TiO_2 particles, [3] *Particle Fibre Toxicol*., **3**, 2006.
U.S.A.	[1] Fisher's least significant difference, [2] Folate status, [3] *Blood*, **92**, 2471-2476, 1988.
U.S.A.	[1] ANOVA followed by Dunnett's method, [2] Nanoparticles, [3] *Toxicol. in vitro,* **19**, 975-983, 2005.
U.S.A.	[1] ANOVA, Dunnett's , Bartlett's test, Kruskal–Wallis or Dunn's test, Levene's test, Shapiro–Wilk test, [2] Kidney bean (*Phaseolus vulgaris)* extract, [3] *Food Chem. Toxicol*., **45**, 32-40, 2007.

Table - 2: Grouping the studies in clusters

Cluster	Statistical tools used
1	The parametric data were analyzed by Dunnett' test and the nonparametric data were by Dunnett type rank sum test or Dunn's multiple comparison test.
2	The parametric data were analyzed by Dunnett' or Scheffe' tests. The nonparametric data were analyzed by Dunnett type rank sum test.
3	After carrying out ANOVA or the data were directly subjected to Dunnett's, Duncan's, Student's or Mann-Whitney tests.
4	The homogeneity was examined by Levene's test, which has of low detection power. Data were also examined for normality

misinterpretation of the data. This may have tremendous negative impact in assessing the safety of the chemical, as the regulatory bodies heavily rely on these data for assessing the safety.

We made an attempt to compare the statistical tools used in 45 countries for analysing the quantitative data obtained from 127 repeated dose toxicity studies with rodents, and found that the tools

Table - 3: Number of studies subjected to homogeneity and/or normality tests

Test for homogeneity or normality	No. of studies
Levene's homogeneity test+Shapiro-Wilks	4
Levene's homogeneity test	2
Shapiro-Wilks test	1
Kolmogorov-Smirnov's test	2

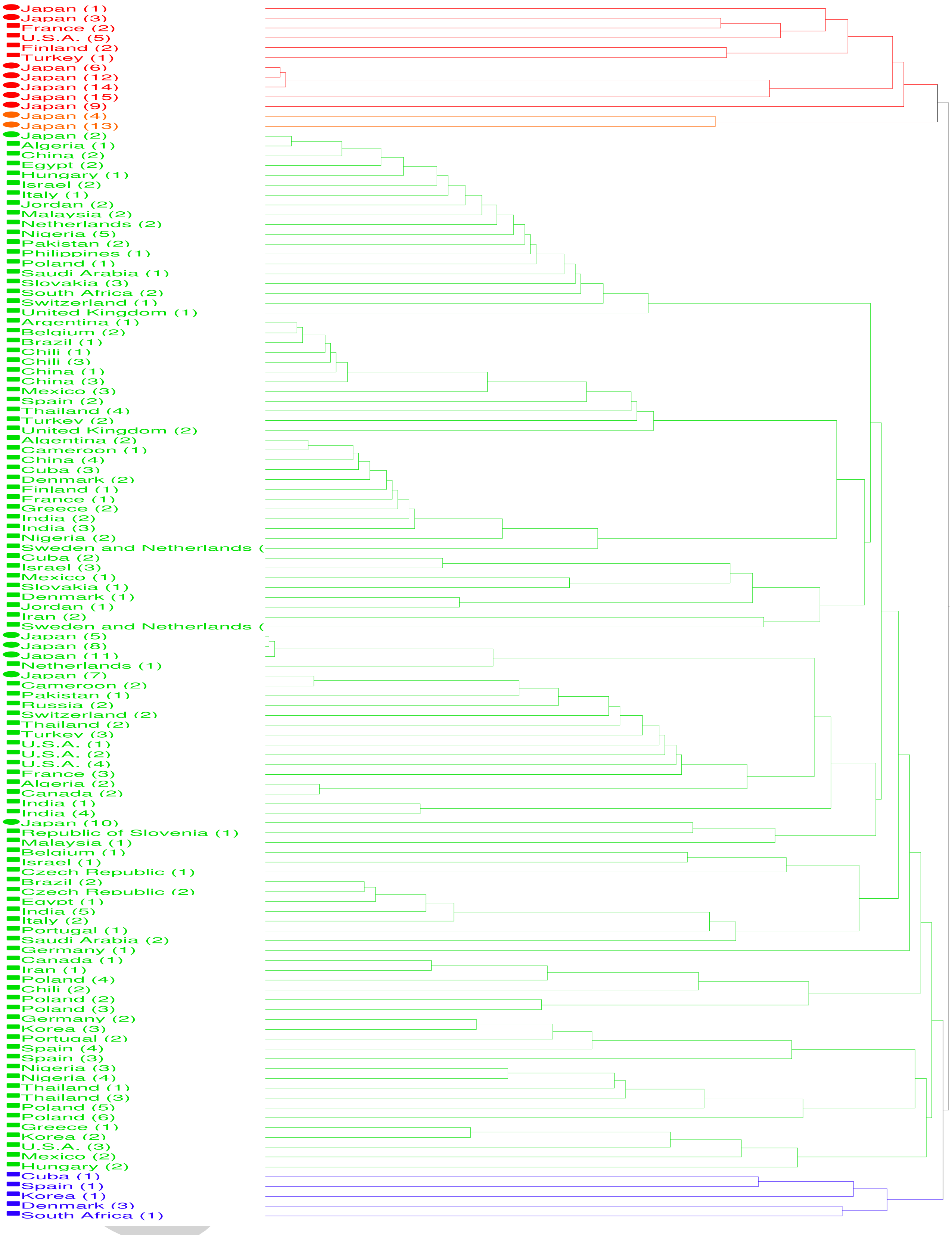

Fig. 1: Classification of statistical analysis methods by cluster analysis. Note: 11 studies in cluster 1 (red), 2 studies in cluster 2 (orange), 109 studies in cluster 3 (green) and 5 studies in cluster 4 (blue)

used were not similar. For example, to analyse the data obtained from repeated dose toxicity studies with rodents, Scheffé's multiple range parametric and non-parametric and Dunnett type (joint type Dunnett) tests are commonly used in Japan (Sakaki *et al*., 2000), but in the other countries use of these tools is not so common. However, statistical techniques used for testing the data obtained from these studies for homogeneity of variance and inter-group comparisons do not differ much between Japan and the other countries. In most of the countries investigated, including Japan, the data are not tested for normality. In Japan, the analysis is usually carried out as per a decision tree (Hamada *et al*., 1998; Kobayashi, 2000; Kobayashi *et al*., 2000).

The data of the repeated dose toxicity studies with rodents for the present investigation are from 45 countries and were obtained from internet.

Investigational materials and analytical method: Statistical methods used in the 45 countries to analyse the data obtained from repeated dose toxicity studies with rodents are given in Table 1. Based on the statistical tools used, these studies were grouped in 4 clusters as given in Table 2 and were subjected to cluster analysis (SAS JMP, Ver. 5, USA). For the cluster analysis, an input of '0' was given, when a statistical tool was not used and '1' was given, when it was used.

Results and Discussion

The classification of statistical tools used in the 45 countries for analyzing data obtained from repeated dose toxicity studies with rodents by cluster analysis is given in Fig. 1. As per the analysis, 11 studies are grouped in cluster 1, 2 in cluster 2, 109 in cluster 3 and 6 studies are grouped in cluster 4. The detection power of statistical tools grouped in cluster 1 for finding significant difference among the groups is extremely low. If the variance of the groups is unequal, using the statistical tools of this cluster may not show a significant difference in the low dose group. The statistical tools of cluster 2 is close to cluster 1, hence the detection power of this cluster is similar to that of cluster 1. If the number of animals is different in the groups, which is usually seen in repeated dose toxicity studies, the detection power of the statistical tools of this cluster for finding a significant difference is further decreased. The statistical tools of cluster 3, which has high detection power, is commonly used in most of the countries. In cluster 4, statistical tools having high detection power were used to examine both homogeneity of variance and normality.

Seven studies from Japan are grouped in cluster 1 of 11 analytical tools, 2 are grouped in cluster 2 of 2 analytical tools and 6 are grouped in cluster 3 of 109 analytical tools. No study from Japan is placed in cluster 4.

Bartlett's test was used to examine homogeneity of variance in studies conducted in most of the countries. However, 6 studies used Levene's test to examine homogeneity of variance, which has less power compared to Bartlett's test. Shapiro-Wilks and Kolmogorov-Smirnov tests were used in two studies each. Interestingly, statistical tool used for *post hoc* comparison was not mentioned in 14 studies (Table 3).

The number of animals in the group can greatly influence outcome of the statistical analysis of the study. It is also common to encounter mortality in repeated dose toxicity studies, which results in difference in number of animals among the groups. In such situation, the selected statistical tool may have low power for detecting a significant difference, hence cannot bring out biologically relevant information. Hence, the number of animals to be used in a group in repeated dose toxicity studies may be decided taking into consideration of the death that could occur in such studies. Bartlett's test is a very sensitive test for testing homogeneity of variance of the data and was used in most of the countries investigated. A slight heterogeneity in variance of the data in one group may result in heterogeneity in variance in the data of all the groups by Bartlett's test, compelling the data to be subjected to a less sensitive non-parametric test. Therefore, for testing homogeneity of variance, Levene's test, which has low sensitivity (Kobayashi *et al*., 1999) may be more appropriate than the Bartlett's test. Present investigation reveals that the data were examined only in 6 studies for both normality and homogeneity of variance. Ideally, the data may be examined for both normality and homogeneity of variance (Kobayashi *et al*., 2008). We suggest Levene's test for testing homogeneity of variance of the data. If the homogeneity of the variance of the groups are not statistically different, we recommend Dunnett's test, and Steel's test, if it is different.

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