number of corpora lutea has not been reported so far for the other PFAAs (ATSDR, 2009; Luebker *et al.,* 2005a). Ovarian follicle development is known to be regulated by the hypothalamic-pituitary-ovarian axis; however, the present result that no effects were found on estrous cyclicity, on the copulation, fertility or delivery index, or on the weight and histopathology of male and female reproductive and endocrine organs denies the possibility that PFOdA affected the axis. Since the recent studies indicate that many other intra-ovarian signaling cascades affect follicular development (Richards and Pangas, 2010), PFOdA might act on such cascades.

PFOdA administration slightly decreased the delivery, live birth and viability index at 1,000 mg/kg/day, and the values were outside the normal range for this strain of rat in the laboratory that performed this study (historical control range for the last twelve years: 84.5-97.0%, 97.7-100.0% and 96.2-100%, respectively). Further, the birth weight of pups was decreased and postnatal body weight gain was inhibited at 1,000 mg/kg/day. Such effects on prenatal and postnatal development could be attributed to secondary effects due to maternal toxicity such as inhibition of body weight gain, but the lipophilic property of PFOdA also indicates the possibility that it was transferred via placenta and/or breast milk and affected the fetuses/pups directly. Previous studies demonstrated developmental effects of the other PFAAs, which were observed even at doses which produced no maternal toxicity (ATSDR, 2009; Butenhoff *et al.,* 2004; Case *et al.,* 2001; Das *et al.,* 2008; Harris and Birnbaum, 1989; Lau *et al.,* 2006, 2003; Luebker *et al.,* 2005a, 2005b; Thibodeaux *et al.,* 2003). Abbott *et al.* (2007) reported an increase in the incidence of full litter loss, reduction of neonatal survival and body weight gain and delayed eye opening in mice given PFOA during days 1-17 of gestation at 0.6 mg/kg/day and above. Interestingly, such developmental effects were not detected in PPARα knockout mice given the same dose. Investigating the PPARα agonistic activity of PFOdA might provide useful information to understand the mechanism of the developmental effects as well as hepatotoxicity.

In summary, oral gavage administration of PFOdA primarily affected the liver, causing centrilobular hepatocyte hypertrophy and necrosis. Other effects included inhibition of body weight gain, anemia, prolongation of APTT and decreased pancreatic zymogen granules. These toxic effects observed at the end of the 42- to 56-day administration period were also detected after the 14-day recovery period. PFOdA also showed reproductive/developmental toxicity: the number of corpora lutea and implantation, total number of pups born, the number of live pups and birth weight of pups were decreased, and the postnatal body weight gain was inhibited. Based on these findings, the NOAEL of PFOdA was considered to be 40 mg/kg/day for repeated dose toxicity and 200 mg/kg/day for reproductive/developmental toxicity.

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REFERENCES

3M (year not specified): Information about PFOS and PFOA. Available at: http://solutions.3m.com/wps/portal/3M/en_US/PFOS/PFOA. Accessed on July 15, 2011.

Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J. and Lau, C. (2007): Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. Toxicol. Sci., **98**, 571-581.

ATSDR (2009): Toxicological Profile for Perfluoroalkyls (Draft for Public Comment), US Department of health and human services, Public health service, Agency for Toxic Substances and Disease Registry (ATSDR). Available at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237. Accessed on July 19, 2011.

Butenhoff, J.L., Kennedy, G.L.Jr., Frame, S.R., O'Connor, J.C. and York, R.G. (2004): The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology, **196**, 95-116.

Canada, E. (2010): Perfluorooctane sulfonate (PFOS), its salts and its precursors. Available at: http://www.ec.gc.ca/toxiques-toxics/Default.asp?lang=En&n=98E80CC6-1&xml=ECD5A576-CEE5-49C7-B26A-88007131860D. Accessed on July 15, 2011.

Case, M.T., York, R.G. and Christian, M.S. (2001): Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. Int. J. Toxicol., **20**, 101-109.

Chang, S.C., Das, K., Ehresman, D.J., Ellefson, M.E., Gorman, G.S., Hart, J.A., Noker, P.E., Tan, Y.M., Lieder, P.H., Lau, C., Olsen, G.W. and Butenhoff, J.L. (2008): Comparative pharmacokinetics of perfluorobutyrate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. Toxicol. Sci., **104**, 40-53.

Chengelis, C.P., Kirkpatrick, J.B., Radovsky, A. and Shinohara, M. (2009): A 90-day repeated dose oral (gavage) toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and motor activity determinations). Reprod. Toxicol., **27**, 342-351.

Das, K.P., Grey, B.E., Zehr, R.D., Wood, C.R., Butenhoff, J.L., Chang, S.C., Ehresman, D.J., Tan, Y.M. and Lau, C. (2008): Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol. Sci., **105**, 173-181.

EU (2006): Directive 2006/122/ECOF. The European Parliament and of the Council of 12 December 2006, amending for the 30th time Council Directive 76/769/EEC on the approximation of the

laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates). Official Journal of the European Union (EU). Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:372:0032:0034:en:PDF. Accessed on July 15, 2011.

Fang, X., Zhang, L., Feng, Y., Zhao, Y. and Dai, J. (2008): Immunotoxic effects of perfluorononanoic acid on BALB/c mice. Toxicol. Sci., **105**, 312-321.

Goldenthal, E.I. (1978): Final report: Ninety day subacute rat toxicity study on Fluorad® Fluorochemical FC-143. International Research and Development Corporation. Study No. 137-089. 3M Reference No. T-3141. US EPA AR226-0441, cited in US EPA (2005).

Goldenthal, E.I., Jessup, D.C., Geil, R.G., Jefferson, N.D. and Arceo, R.J. (1978): Ninety-day subacute rat study. Study No. 137-085. International Research and Development Corporation, cited in OECD (2002).

Griffith, F.D. and Long, J.E. (1980): Animal toxicity studies with ammonium perfluorooctanoate. Am. Ind. Hyg. Assoc. J., **41**, 576-583.

Harris, M.W. and Birnbaum, L.S. (1989): Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam. Appl. Toxicol., **12**, 442-448.

Hekster, F.M., Laane, R.W. and de Voogt, P. (2003): Environmental and toxicity effects of perfluoroalkylated substances. Rev. Environ. Contam. Toxicol., **179**, 99-121.

Ioannidis, O., Lavrentieva, A. and Botsios, D. (2008): Nutrition support in acute pancreatitis. JOP, **9**, 375-390.

Japanese Animal Welfare Law (2005): Act on Welfare and Management of Animals. Act No. 105 of October 1, 1973. As amended up to Act No. 68 of June 22, 2005.

Kawashima, Y., Kobayashi, H., Miura, H. and Kozuka, H. (1995): Characterization of hepatic responses of rat to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. Toxicology, **99**, 169-178.

Kemper, R.A. (2003): Perfluorooctanoic acid: toxicokinetics in the rat. Association of plastics manufactures of Europe. Submitted to the US EPA's admistrative record. AR226-1499, cited in ASTDR (2009).

Kennedy, G.L.Jr. (1987): Increase in mouse liver weight following feeding of ammonium perfluorooctanoate and related fluorochemicals. Toxicol. Lett., **39**, 295-300.

Kudo, N., Bandai, N., Suzuki, E., Katakura, M. and Kawashima, Y. (2000): Induction by perfluorinated fatty acids with different carbon chain length of peroxisomal beta-oxidation in the liver of rats. Chem. Biol. Interact., **124**, 119-132.

Kudo, N., Katakura, M., Sato, Y. and Kawashima, Y. (2002): Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem. Biol. Interact., **139**, 301-316.

Kudo, N., Suzuki-Nakajima, E., Mitsumoto, A. and Kawashima, Y. (2006): Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice:in relation to induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1-acylglycerophosphocholine acyltransferase. Biol. Pharm. Bull., **29**, 1952-1957.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A. and Seed, J. (2007): Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci., **99**, 366-394.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B. and Strynar, M.J. (2006): Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci., **90**, 510-518.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L. and Stevenson, L.A. (2003): Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol. Sci., **74**, 382-392.

Lieder, P.H., Chang, S.C., York, R.G. and Butenhoff, J.L. (2009a): Toxicological evaluation of potassium perfluorobutanesulfonate in a 90-day oral gavage study with Sprague-Dawley rats. Toxicology, **255**, 45-52.

Lieder, P.H., York, R.G., Hakes, D.C., Chang, S.C. and Butenhoff, J.L. (2009b): A two-generation oral gavage reproduction study with potassium perfluorobutanesulfonate (K+PFBS) in Sprague Dawley rats. Toxicology, **259**, 33-45.

Luebker, D.J., Case, M.T., York, R.G., Moore, J.A., Hansen, K.J. and Butenhoff, J.L. (2005a): Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology, **215**, 126-148.

Luebker, D.J., York, R.G., Hansen, K.J., Moore, J.A. and Butenhoff, J.L. (2005b): Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology, **215**, 149-169.

Martin, J.W., Mabury, S.A., Solomon, K.R. and Muir, D.C. (2003): Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem., **22**, 196-204.

ME (2006): Standards Relating to the Care, Management of Laboratory Animals and Relief of Pain. Announcement No. 88 of Ministry of the Environment (ME), Japan, dated April 28, 2006.

ME (2008) 19. Perfluorooctane sulfonic acid and its salt forms (Perfluorooctane sulfonate: PFOS, in Japanese). Initial environmental risk assessment of chemicals, Vol.6, Ministry of the Environment (ME). Available at: http://www.env.go.jp/chemi/report/h19-03/pdf/chpt1/1-2-2-19.pdf. Accesed on July 27, 2011.

ME, METI and MHLW (2008): Standard concerning testing laboratories implementing tests for new chemical substances etc.. Joint notification by director generals of Environmental Policy Bureau, Ministry of the Environment (ME), Japan (Kanpokihatsu No. 031121004) and Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry (METI), Japan (Seikyokuhatsu No. 3), dated November 17, 2003 and by director general of Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (MHLW), Japan (Yakusyokuhatsu No. 1121003), dated November 21, 2003. As amended up to July 4, 2008.

Mertens, J.J., Sved, D.W., Marit, G.B., Myers, N.R., Stetson, P.L., Murphy, S.R., Schmit, B., Shinohara, M. and Farr, C.H. (2010): Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. Int. J. Toxicol., **29**, 358-371.

MHLW, ME and NITE (year not specified): Japan CHEmicals Collaborative Knowledge database (J-CHECK, in Japanese). Ministry of Health, Labour and Welfare (MHLW), Ministry of the Environment (ME) and National Institute of Technology and Evaluation (NITE), Japan. Available at: http://www.safe.nite.go.jp/jcheck/Top.do;jsessionid=70E9B212CA59B38B6F8FC6B4BF47FEDD. Accessed on July 15, 2011.

OECD (1996): Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Section 4: Health Effects, Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. Adopted on 22 March, 1996.

OECD (2002): Hazard assessment of perfluorooctane sulfonate

(PFOS) and its salts. Organisation for Economic Co-operation and Development (OECD). ENV/JM/RD(2002)17/FINAL. Available at: http://www.oecd.org/dataoecd/23/18/2382880.pdf. Accessed on July 15, 2011. .

OECD (2007): Report of an OECD workshop on perfluorocarbosylic acids (PFCAs) and precursors. Organisation for Economic Co-operation and Development (OECD), Environment directorate, Joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology. Available at: http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2007)11&doclanguage=en. Accessed on Juny 15, 2011.

Ohmori, K., Kudo, N., Katayama, K. and Kawashima, Y. (2003): Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology, **184**, 135-140.

Ove, P., Coetzee, M.L., Chen, J. and Morris, H.P. (1972): Differences in synthesis and degradation of serum proteins in normal and hepatoma-bearing animals. Cancer Res., **32**, 2510-2518.

Perkins, R.G., Butenhoff, J.L., Kennedy, G.L.Jr. and Palazzolo, M.J. (2004): 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug Chem. Toxicol., **27**, 361-378.

Permadi, H., Lundgren, B., Andersson, K. and DePierre, J.W. (1992): Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes, enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. Biochem. Pharmacol., **44**, 1183-1191.

Permadi, H., Lundgren, B., Andersson, K., Sundberg, C. and DePierre, J.W. (1993): Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica, **23**, 761-770.

Richards, J.S. and Pangas, S.A. (2010) The ovary: basic biology and clinical implications. J. Clin. Invest., **120**, 963-972.

Schultz, M.M., Barofsky, D.F. and Field, J.A. (2003): Fluorinated Alkyl Surfactants. Environ. Eng. Sci., **20**, 487-501.

Shi, Z., Zhang, H., Liu, Y., Xu, M. and Dai, J. (2007): Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. Toxicol. Sci., **98**, 206-215.

Sibinski, L.J. (1987): Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. Vol.1-4, 3M Company/RIKER Exp. No.0281CR0012; 8EHQ-1087-0394, cited in US EPA (2005).

Stockholm Convention (2010): The 9 new POPs under the Stockholm Convention. Stockholm Convention on Persistent Organic Pollutants (POPs). Available at: http://chm.pops.int/Programmes/New%20POPs/The%209%20new%20POPs/tabid/672/language/en-GB/Default.aspx. Accessed on July 15, 2011.

Stump, D.G., Holson, J.F., Murphy, S.R., Farr, C.H., Schmit, B. and Shinohara, M. (2008): An oral two-generation reproductive toxicity study of S-111-S-WB in rats. Reprod. Toxicol., **25**, 7-20.

Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Barbee, B.D., Richards, J.H., Butenhoff, J.L., Stevenson, L.A. and Lau, C. (2003): Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. Toxicol. Sci., **74**, 369-381.

Thomford, P.J. (2002): Final report: 104-seek dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. Covance study No. 6329-183, Covence Laboratories Inc. US EPA AR226-1070a, AR226-0956, cited in OECD (2002) and ME (2008).

US EPA (2000): EPA and 3M Announce Phase Out of PFOS. News Release By Date. United States Environmental Protection Agency (US EPA). May 16 2000. Available at: http://yosemite.epa.gov/opa/admpress.nsf/0/33aa946e6cb11f35852568e1005246b4?opendocument. Accessed on July 15, 2011.

US EPA (2005): Draft risk assessment of the potential human health effects associated with exposure to perfluorooctanoic acid and its salts. United States Environmental Protection Agency (EPA), Office of Pollution Prevention and Toxics, Risk Assessment Division. Available at: http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.pdf. Accessed on July 19, 2011.

US EPA (2008a): Perfluorooctanoic acid (PFOA) and fluorinated telomers. United States Environmental Protection Agency (US EPA). Available at: http://www.epa.gov/oppt/pfoa/. Accessed on July 15, 2011. .

US EPA (2008b): Significant new uses of chemical substances. United States Environmental Protection Agency (US EPA). Code of Federal Regulations. 40 CFR 721. Available at: http://www.gpo.gov/fdsys/pkg/CFR-2008-title40-vol30/pdf/CFR-2008-title40-vol30-part721.pdf. Accessed on July 15, 2011. .

van Otterdijk, F.M. (2007a): Repeated dose 28-day oral toxicity study with MTDID-8391 by daily gavage in the rat, followed by a 21-day recovery. NOTOX Project 470677. Available at: http://www.health.state.mn.us/divs/eh/hazardous/28daymaintext.pdf. Accessed on July 19, 2011.

van Otterdijk, F.M. (2007b): Repeated dose 90-day oral toxicity study with MTDID 8391 by daily gavage in the rat, followed by a 3-week recovery, NOTOX Project 484492. Available at http://www.health.state.mn.us/divs/eh/hazardous/90daypfbareport.pdf. Accessed on July 19, 2011.

Xie, Y., Yang, Q., Nelson, B.D. and DePierre, J.W. (2003): The relationship between liver peroxisome proliferation and adipose tissue atrophy induced by peroxisome proliferator exposure and withdrawal in mice. Biochem. Pharmacol., **66**, 749-756.

Yang, Q., Xie, Y., Alexson, S.E., Nelson, B.D. and DePierre, J.W. (2002): Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochem. Pharmacol., **63**, 1893-1900.

Yang, Q., Xie, Y. and Depierre, J.W. (2000): Effects of peroxisome proliferators on the thymus and spleen of mice. Clin. Exp. Immunol., **122**, 219-226.

Zhang, H., Shi, Z., Liu, Y., Wei, Y. and Dai, J. (2008): Lipid homeostasis and oxidative stress in the liver of male rats exposed to perfluorododecanoic acid. Toxicol. Appl. Pharmacol., **227**, 16-25.

【報文】

OECD 化学物質対策の動向（第 17 報）

－第 28 回 OECD 高生産量化学物質初期評価会議（2009 年パリ）

Progress on OECD Chemicals Programme (17) – SIAM 28 in Paris, 2009

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要旨：第 28 回 OECD 高生産量化学物質初期評価会議（SIAM 28）が 2009 年 4 月にフランス・パリで開催され、日本が担当した 3 物質（2-(1-メチルエトキシ)エタノール：CAS 番号 109-59-1、2-(2'-ヒドロキシ-3'-*tert*-ブチル-5'-メチルフェニル)-5-クロロベンゾトリアゾール（別名ブメトリゾール）：CAS 番号 3896-11-5、C.I.フルオレセントブライトナー271：CAS 番号 41267-43-0）の SIAP について合意が得られた。本稿では本会議で合意の得られたこれら 3 物質の初期評価文書について紹介する。
キーワード：OECD、HPV プログラム、SIDS 初期評価会議

Abstract: The 28th Screening Information Data Set (SIDS) Initial Assessment Meeting (SIAM 28) was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France. The initial assessment documents of three substances, 2-(1-methylethoxy)ethanol (CAS number: 109-59-1), 2-*tert*-butyl-6- (5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (CAS number: 3896-11-5) and Fluorescent-271 (CAS number: 41267-43-0) were submitted by the Japanese Government with or without the collaboration with ICCA. These SIDS Initial Assessment Profiles (SIAPs) of the substances were agreed at the meeting. In this report, the documents of these substances are introduced.
Keywords: OECD, HPV programme, SIDS Initial Assessment Meeting

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1　はじめに

経済協力開発機構（Organisation for Economic Co-operation and Development : OECD）では、1992 年に始まった高生産量化学物質点検プログラム（High Production Volume Chemical (HPV) Programme）により、加盟各国における高生産量化学物質の安全性の評価を行っている（長谷川ら 1999a、江馬 2006）。日本政府は初回より評価文書を提出しており、2001 年からは国際化学工業協会協議会(International Council of Chemical Associations : ICCA）の参画に伴い日本化学工業協会加盟企業も評価文書の原案作成に参加している。第 27 回までの初期評価会議（Screening Information Data Set (SIDS) Initial Assessment Meeting : SIAM）において日本政府が担当し結論および勧告が合意された化学物質の評価文書のヒト健康影響または環境影響・曝露情報部分については既に紹介してきた（長谷川ら 1999b、2000、2001；高橋ら 2004、2005a、2005b、2006a、2006b、2006c、2007a、2007b、2007c、2008、2009、2010）。また、第 1 回 SIAM（SIAM 1）から SIAM 18 までの結果の概要および SIAM 19 から SIAM 28 の各会議内容についても紹介してきた（松本ら 2005a、2005b、2006a、2006b、2007a、2007b、2007c、2008a、2008b、2009a、2009b）。

本稿では SIAM 28 で合意に至った日本担当物質の評価文書の概要を紹介する。なお、OECD ガイドラインに則した毒性試験についてはガイドライン番号を示したが、遺伝毒性に関しては 1 物質に対して多種の試験が行われることもあり、結果のみ簡潔に示すこととした。

2　SIAM 28 で合意された日本担当物質の初期評価内容

2009 年 4 月にパリ（フランス）で開催された SIAM 28 において、我が国は 3 物質の初期評価文書を提出し、それら全ての初期評価結果および勧告が合意された。

SIAM における合意は FW（The chemical is a candidate for further work.）または LP（The chemical is currently of low priority for further work.）として示されている。FW は「追加の調査研究作業が必要である」、LP は「現状の使用状況においては追加作業の必要はない」ことを示す。

（1）2-(1-メチルエトキシ)エタノール

英名 2-(1-Methylethoxy)ethanol (109-59-1)（日本政府）

1）曝露状況

本物質は塗装工程における溶剤として使用される。本物質は閉鎖系で製造されるため職業曝露の可能性は低いが、使用時には吸入または経皮曝露の可能性がある。また、工業的利用以外、消費者用製品として販売されていないので、消費者曝露の可能性は低い。本物質の製造過程や使用による廃水は適切に処理されているので、環境への排出は少ない。

2）環境影響

本物質が大気相・土壌相・水相に放出された場合は主に土壌相（51.4%）と水相（47.0%）に分布することが予測された。本物質は容易に生分解されないが、親油性が低いため、魚類への生物濃縮性は低いと推定された（BCF : 3.16 [計算値]）。

水生生物に対する急性毒性について、魚類の半数致死濃度（LC_{50}）は 100 mg/L以上（96 時間、OECD TG 203）、ミジンコの半数影響濃度（EC_{50}）は 970 mg/L以上（48 時間、遊泳阻害、OECD TG 202）、藻類のEC_{50}は 1,000 mg/L以上（72 時間、生長阻害（速度法）：

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OECD TG 201）であった。慢性毒性については、ミジンコの最大無影響濃度（NOEC）は98 mg/L（21日間、繁殖阻害：OECD TG 211)、藻類のNOECは1,000 mg/L（72時間、生長阻害（速度法）：OECD TG 201）であった。

3）健康影響

本物質はラットへの腹腔内投与後、代謝されてIsopropoxyacetic acid（30%)、N-Isopropoxyacetylglycine（46%)、Ethylene glycol（13%）となり、主に尿中へ24時間以内に排泄された。イヌでも同様の代謝プロファイルが認められた。

ラットの単回経口投与毒性試験（OECD TG 401）において最高用量2,000 mg/kg bwでも死亡例は認められず、LD_{50}は雌雄ともに2,000 mg/kg bw以上であった。一般状態として2,000 mg/kg bwで一過性の赤色尿、排便量の減少、および体重の低値が認められた。

本物質にはウサギの皮膚に対する刺激性が認められた。

ラットに0、30、125または500 mg/kg bw/dayの本物質を強制経口投与した28日間反復経口投与毒性試験（OECD TG 407）において、死亡例はみられなかった。500 mg/kg bw/dayの雌雄において、尿潜血およびビリルビン、血液学検査での貧血様変化が認められ、また、骨髄像所見での造血亢進、脾臓の重量高値・組織学的変化、骨髄の組織学的変化が認められた。125 mg/kg bw/dayの雌雄でも、血液学検査における貧血様変化、脾臓の重量高値（雌)・組織学的変化が認められた。さらに、30 mg/kg bw/dayの雌雄で骨髄像所見における造血亢進が認められた。これらのことから反復経口投与毒性のLOAELは30 mg/kg bw/dayとされた。

雌雄ラットに交配前2週間から交配期間を含め、雄では48日間、雌では分娩後哺育3日まで（41～47日間)、0、8、30または125 mg/kg bw/dayを強制経口投与した経口投与簡易生殖毒性試験（OECD TG 421）において、親動物では30 mg/kg bw/day以上の雌で赤色尿がみられ、125 mg/kg bw/dayでは雄に赤色尿、雌雄の脾臓重量の高値、雄の脾臓で髄外造血・色素沈着が認められた。一方、生殖能および児の発生・発育については本物質投与に影響は認められなかった。これらのことから、反復経口投与毒性のNOAELは雄で30 mg/kg bw/day、雌で8 mg/kg bw/day、生殖発生毒性のNOAELは125 mg/kg bw/dayとされた。

雌雄ラットに0、142、441、または891 ppm（0、0.61、1.90、3.83 mg/L）[追加試験：0、10、30、100 ppm（0、0.04、0.13、0.43 mg/L）]を4週間（5日/週、6時間/日）吸入曝露（全身）させた反復吸入毒性試験（OECD TG 412）において、100 ppm以上で溶血性貧血、142 ppm以上で脾臓に髄外造血・色素沈着、441 ppm以上では脾臓重量の高値が認められ、反復吸入投与毒性のNOAECは30 ppm（0.13 mg/L）とされた。

雌雄のラット・ウサギ・モルモット・イヌに0、25、50、または200 ppm（0、0.1075、0.215、0.86 mg/L）を26週間（5日/週、6時間/日）吸入曝露（全身）させた吸入毒性試験では、ウサギ・モルモット・イヌに毒性影響は認められなかった。ラットでは、25 ppm以上で雌雄の赤血球に溶血の起因となる浸透圧抵抗性の低下が認められ、50 ppm以上で雌雄の脾臓にヘモジデリン沈着が認められた。さらに、200 ppmでは雌雄のヘモグロビン濃度・ヘマトクリット値の低値、平均赤血球容積・脾臓重量の高値、脾臓における髄外造血が認められ、雌の肝臓における褐色色素の増加、雄の肝実質における少量の脂肪も認められた。これらのことから、反復吸入投与毒性のLOAECは25 ppm（0.1075 mg/L）とされた。

細菌を用いる復帰突然変異試験およびチャイニーズ・ハムスター培養細胞を用いる染色体異常試験はS9mixの存在/非存在下で陰性であった。

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4）結論と勧告

本物質は健康に対して有害性（皮膚刺激性、反復投与毒性－溶血作用・骨髄毒性）を示すが、現況において十分なリスク管理がなされているので、健康影響についてLPと勧告された。環境に対しては有害性が低いので、環境影響についてLPと勧告された。

（2）2-(2'-ヒドロキシ-3'-*tert*-ブチル-5'-メチルフェニル)-5-クロロベンゾトリアゾール（別名ブメトリゾール）

英名 2-*tert*-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol（3896-11-5）
（日本政府、原案作成：ICCA日本企業）

1）曝露状況

本物質は、建築材料や自動車内装部品に使用されるインク・塗料・シーラント・プラスチック（主にポリオレフィンやポリエステル）のUV吸収剤として用いられる。その他、化粧品や芳香剤等の個人ケア製品のUVフィルターとして使用され、さらに、EU、日本、米国では食品と接触する物質に使用できる添加物として認可されている。本物質には吸入や皮膚接触による職業曝露の可能性がある。消費者曝露としては主に皮膚接触の可能性があり、さらに、包装から食品に移った本物質を経口摂取することも考えられる。本物質は水溶性が低いので、本物質を含む製品の廃棄による環境への排出はわずかであるが、本物質を含む個人ケア製品の使用により、廃水として環境に排出される可能性がある。

2）環境影響

予測の結果、本物質が大気相・土壌相・水相に放出された場合は土壌相（74.4%）、底質相（19.5%）、水中（6.1%）に分布し、大気相に放出された場合は土壌相（93.7%）、底質相（4.8%）に分布し、水相に放出された場合は底質相（76.3%）、水相（23.7%）に分布し、土壌相に放出された場合は主に土壌相（99.9%）に留まるとされた。本物質は容易に生分解されず、また、魚類への生物濃縮性は比較的高い（OECD TG305c、BCF：196-802（0.05 mg/L）、548-895（0.005 mg/L））。

水生生物に対する急性毒性について、魚類のLC_{50}（96時間、OECD TG 203）、ミジンコのEC_{50}（24時間、遊泳阻害、OECD TG 202）、藻類のEC_{50}（72時間、生長阻害（面積法））はともに水溶解度（1.0 mg/L未満、20℃）以上であった。また、活性汚泥微生物の半数呼吸阻害濃度（IC_{50}）は100 mg/L以上（3時間、OECD TG 209）であった。慢性毒性については、藻類のNOEC（72時間、生長阻害（面積法））が水溶解度以上であった。

3）健康影響

急性吸入毒性試験において到達可能な最高粉体濃度でも死亡例は認められず、LC_{50}は最高粉体濃度の270 mg/m^{3}以上（4時間、ラット）であった。急性経口毒性試験（OECD TG 423）において最高用量でも死亡例は認められず、LD_{50}は2,000 mg/kg bw以上（ラット）であった。

本物質には皮膚や眼に対する刺激性はない。本物質はモルモットにおいて皮膚感作性を示さず（OECD TG406）、ヒトにおいても皮膚感作性を示さなかった。また、モルモットにおける光アレルギー性も認められない（OECDドラフトガイドライン）。

13週間、イヌに0、200、1,000、または5,000 ppm（雄：0、6.2、29.6、168 mg/kg bw/day、雌：0、6.5、32.2、153 mg/kg bw/day）を混餌投与した反復経口毒性試験において、雌の5,000 ppmで体重の低値が認められたことから、NOAELは1,000 ppm（雄：**29.6 mg/kg bw/day**、雌：**32.2 mg/kg bw/day**）とされた。

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104 週間、ラットに 0、1,000、3,000、または 10,000 ppm（雄：0、37.7、113.2、382.6 mg/kg bw/day、雌：0、50.4、147.7、501.9 mg/kg bw/day）を混餌投与した反復経口毒性試験（OECD TG 453 相当）において、10,000 ppmで雌雄に赤血球パラメーターの低値、雄に体重増加抑制が認められたことから、NOAELは 3,000 ppm（雄：113.2 mg/kg bw/day、雌：147.7 mg/kg bw/day）とされた。

24 ヶ月間、マウスに 0、5、50、または 500 mg/kg feed（雄：0、0.7、6、62 mg/kg bw/day、雌：0、0.7、6、59 mg/kg bw/day）を混餌投与した反復経口毒性試験（OECD TG 451 相当）において、最高用量でも毒性影響は認められず、NOAELは 500 mg/kg feed（雄：62 mg/kg bw/day、雌：59 mg/kg bw/day）とされた。

ラットに交配前 2 週間および交配期間を含め、雄では計 42 日間、雌では分娩後哺育 6 日まで（44～56 日間）、0、62.5、250 及び 1,000 mg/kg bw/dayを強制経口投与した反復投与毒性・生殖発生毒性併合試験（OECD TG 422）において、本物質投与による毒性影響は認められなかったので、反復投与毒性のNOAELは雌雄ともに1000 mg/kg bw/dayとされた。また、生殖発生に関する毒性影響も認められず、生殖発生毒性のNOAELは1,000 mg/kg bw/dayとされた。

妊娠 6-15 日の雌ラットに 0、300、1,000 または 3,000 mg/kg bw/dayを強制経口投与した出生前発生毒性試験（OECD TG 414 相当）では、最高用量まで母体および胎児に投与の影響は認められず、発生毒性のNOAELは3,000 mg/kg bw/dayとされた。

妊娠 6-15 日の雌マウスに 0、300、1,000 または 3,000 mg/kg bw/dayを強制経口投与した出生前発生毒性試験（OECD TG 414 相当）では、3,000 mg/kg bw/dayの胎児において平均重量が高いにもかかわらず胸骨分節の骨化遅延が認められたが、催奇形性は認められなかった。また、最高用量まで母体への毒性影響は認められなかった。このことから、発生毒性のNOAELは1,000 mg/kg bw/dayとされた。

細菌を用いる復帰突然変異試験およびチャイニーズ・ハムスター培養細胞を用いる染色体異常試験は S9mix の存在/非存在下で陰性であった。また、*in vivo* では、マウスを用いた優性致死試験で優性致死作用は認められず、チャイニーズ・ハムスターを用いた骨髄染色体異常試験および小核試験でも陰性であった。

104 週間、ラットに 10,000 ppm（雄：382.6 mg/kg bw/day、雌：501.9 mg/kg bw/day）まで混餌投与した試験（前出の反復投与試験と同じ）において発がん性は認められなかった。また、24 ヶ月間、マウスに 500 mg/kg feed/day（雄：62 mg/kg bw/day、雌：59 mg/kg bw/day）まで混餌投与した試験でも発がん性は認められなかった。

4）結論と勧告

本物質は健康に対して有害性が低いので、健康影響について LP と勧告された。また、環境に対しては有害性（低生分解性、比較的高い生物濃縮性）を示すので、環境影響については FW と勧告され、底生生物への慢性毒性試験（OECD TG 218）を行うことが推奨された。

（3）C.I.フルオレセントブライトナー271

英名 Fluorescent-271 (41267-43-0)（日本政府）

1）曝露状況

本物質は製紙用の蛍光増白剤として使用される。本物質は閉鎖系で加工されるため、職業曝露の可能性は低い。紙をリサイクルする際、高濃度での本物質の放出が予測されるが、利用可能な測定データは現在のところない。本物質は製品に含まれるため、皮膚接触によ

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る消費者曝露の可能性がある。

2）環境影響

本物質が大気相・土壌相・水相に放出された場合は主に水相（59.5%）と土壌相（40.3%）に分布することが予測された。本物質は容易に生分解しないが、魚類への生物濃縮性は低いと推定された（BCF：3.16 [計算値]）。

水生生物に対する急性毒性について、魚類のLC_{50}は100 mg/L以上（96時間、OECD TG 203）、ミジンコのEC_{50}は97 mg/L以上（48時間、遊泳阻害、OECD TG 202）、藻類のEC_{50}は23 mg/L以上（72時間、生長阻害（速度法）：OECD TG 201）であった。慢性毒性については、ミジンコのNOECは17 mg/L（21日間、繁殖阻害：OECD TG 211）、藻類のNOECは8.6 mg/L（72時間、生長阻害（速度法）：OECD TG 201）であった。

3）健康影響

雌ラットの単回経口投与毒性試験（OECD TG 423）において最高用量でも死亡例は認められず、LD_{50}は2,000 mg/kg bw以上であった。

ラットに交配前2週間および交配期間を含め、雄では計43日間、雌では分娩後哺育4日まで（41～55日間）、0、20、60及び200 mg/kg bw/dayを強制経口投与した反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では、200 mg/kg bw/dayで雌雄の体重および摂餌量の低値がみられた。雌雄の腎臓において、20 mg/kg bw/day以上で近位尿細管上皮の空胞変性、60 mg/kg bw/day以上で淡色化、200 mg/kg bw/dayで肥大、重量増加が認められた。また、200 mg/kg bw/dayでは腎障害に伴い、貧血や血液生化学検査値および尿検査値の変化が雌雄に認められた。回復期間（14日間）終了時には腎障害の回復が認められた。生殖発生毒性に関しては、親動物の生殖能力への影響は認められなかったが、200 mg/kg bw/dayで哺育0日の児体重低値が認められた。これらより、**反復投与毒性のLOAEL**は雌雄ともに**20 mg/kg bw/day**、**生殖毒性のNOAEL**は最高用量の**200 mg/kg bw/day**、**発生毒性のNOAEL**は**60 mg/kg bw/day**とされた。

細菌を用いる復帰突然変異試験は陰性であった。チャイニーズ・ハムスター培養細胞を用いる染色体異常試験ではS9mix存在下では陰性であったが、S9mix非存在下では陽性であった。

4）結論と勧告

本物質は健康に対して有害性（反復投与による腎毒性、*in vitro*での染色体異常誘発）を示し、また、曝露量の調査が十分ではない。これらのことから、健康影響については FW と勧告され、職業および消費者曝露量の調査が推奨されると共に、*in vivo*での遺伝毒性試験が必要とされた。また、環境に対しては有害性が低いので、環境影響については LP と勧告された。

参考文献：

1) 江馬　眞（2006）：OECDの高生産量化学物質安全性点検プログラムとその実施手順. 化学生物総合管理, 2, 83-103.
2) 高橋美加，平田睦子，松本真理子，広瀬明彦，鎌田栄一，長谷川隆一，江馬　眞（2004）：OECD化学物質対策の動向（第5報）. 国立医薬品食品衛生研究所報告, 122, 37-42.

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3) 高橋美加，平田睦子，松本真理子，広瀬明彦，鎌田栄一，長谷川隆一，江馬　眞 (2005a)：OECD 化学物質対策の動向（第 6 報）．化学生物総合管理, 1, 46-55.
4) 高橋美加，平田睦子，松本真理子，広瀬明彦，鎌田栄一，長谷川隆一，江馬　眞 (2005b)：OECD 化学物質対策の動向（第 7 報）．国立医薬品食品衛生研究所報告, 123, 46-52.
5) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2006a)：OECD 化学物質対策の動向（第 8 報）．化学生物総合管理, 2, 147-162.
6) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2006b)：OECD 化学物質対策の動向（第 9 報）．化学生物総合管理, 2 163-175.
7) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2006c)：OECD 化学物質対策の動向（第 11 報）．国立医薬品食品衛生研究所報告, 124, 62-68.
8) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2007a)：OECD 化学物質対策の動向（第 10 報）．化学生物総合管理, 2 286-301.
9) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2007b)：OECD 化学物質対策の動向（第 12 報）．化学生物総合管理, 3, 43-55.
10) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2007c)：OECD 化学物質対策の動向（第 13 報）．国立医薬品食品衛生研究所報告, 125, 101-106.
11) 高橋美加，松本真理子，川原和三，菅野誠一郎，菅谷芳雄，広瀬明彦，鎌田栄一，江馬　眞 (2008)：OECD 化学物質対策の動向（第 14 報）．化学生物総合管理, 4, 225-236.
12) 高橋美加，松本真理子，宮地繁樹，菅野誠一郎，菅谷芳雄，平田睦子，広瀬明彦，鎌田栄一，江馬　眞 (2009)：OECD 化学物質対策の動向（第 15 報）．化学生物総合管理, 5, 193-200.
13) 高橋美加，松本真理子，宮地繁樹，菅野誠一郎，菅谷芳雄，平田睦子，小野　敦，鎌田栄一，江馬　眞，広瀬明彦 (2010)：OECD 化学物質対策の動向（第 16 報）．化学生物総合管理, 6, 180-188.
14) 長谷川隆一，中館正弘，黒川雄二 (1999a)：OECD 化学物質対策の動向. J. Toxicol. Sci., 24, app. 11-19.
15) 長谷川隆一，鎌田栄一，広瀬明彦，菅野誠一郎，福間康之臣，高月峰夫，中館正弘，黒川雄二 (1999b)：OECD 化学物質対策の動向（第 2 報）．J. Toxicol. Sci., 24, app. 85-92.
16) 長谷川隆一，小泉睦子，鎌田栄一，広瀬明彦，菅野誠一郎，高月峰夫，黒川雄二 (2000)：OECD 化学物質対策の動向（第 3 報）．J. Toxicol. Sci., 25, app. 83-96.
17) 長谷川隆一，小泉睦子，広瀬明彦，菅原尚司，黒川雄二 (2001)：OECD 化学物質対策の動向（第 4 報）．J. Toxicol. Sci., 26, app. 35-41.
18) 松本真理子，田中里依，川原和三，菅谷芳雄，江馬　眞 (2005a)：OECD 高生産量化学物質点検プログラム：第 19 回初期評価会議概要．化学生物総合管理, 1, 280-288.
19) 松本真理子，鈴木理子，川原和三，菅谷芳雄，江馬　眞 (2005b)：OECD 高生産量化学物質点検プログラム：第 20 回初期評価会議概要．化学生物総合管理, 1, 445-453.
20) 松本真理子，高橋美加，平田睦子，広瀬明彦，鎌田栄一，長谷川隆一，江馬　眞 (2006a)：OECD 高生産量化学物質点検プログラム：第 18 回初期評価会議までの概要．化学生物総合管理, 2, 104-134.
21) 松本真理子，川原和三，菅谷芳雄，江馬　眞 (2006b)：OECD 高生産量化学物質点検プログラム：第 21 回初期評価会議概要．化学生物総合管理, 2, 135-146.
22) 松本真理子，日下部哲也，川原和三，菅谷芳雄，江馬　眞 (2007a)：OECD 高生産量化

化学生物総合管理　第 7 巻第 1 号 (2011.6)　47-54 頁
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学物質点検プログラム：第 22 回初期評価会議概要．化学生物総合管理, 2 302-312.
23) 松本真理子，大井恒宏，宮地繁樹，菅谷芳雄，江馬　眞（2007b）：OECD 高生産量化学物質点検プログラム：第 23 回初期評価会議概要．化学生物総合管理, 3, 56-65.
24) 松本真理子，山本展裕，宮地繁樹，菅谷芳雄，江馬　眞（2007c）：OECD 高生産量化学物質点検プログラム：第 24 回初期評価会議概要．化学生物総合管理, 3, 180-189.
25) 松本真理子，山本展裕，宮地繁樹，菅谷芳雄，江馬　眞（2008a）：OECD 高生産量化学物質点検プログラム：第 25 回初期評価会議概要．化学生物総合管理, 4, 136-143.
26) 松本真理子，宮地繁樹，菅谷芳雄，江馬　眞，広瀬明彦（2008b）：OECD 高生産量化学物質点検プログラム：第 26 回初期評価会議概要．化学生物総合管理, 4, 237-245.
27) 松本真理子，宮地繁樹，菅谷芳雄，広瀬明彦（2009a）：OECD 高生産量化学物質点検プログラム：第 27 回初期評価会議概要．化学生物総合管理, 5, 105-115.
28) 松本真理子，宮地繁樹，菅谷芳雄，広瀬明彦（2009b）：OECD 高生産量化学物質点検プログラム：第 28 回初期評価会議概要．化学生物総合管理, 5, 201-209.

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Original Article

Influence of coefficient of variation in determining significant difference of quantitative values obtained from 28-day repeated-dose toxicity studies in rats

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ABSTRACT — In order to understand the influence of coefficient of variation (CV) in determining significant difference of quantitative values of 28-day repeated-dose toxicity studies, we examined 59 parameters of 153 studies conducted in accordance with Chemical Substance Control Law in 12 test facilities. Sex difference was observed in 12 parameters and 10 parameters showed large CV in females. The minimum CV was 0.74% for sodium. CV of electrolytes was comparatively small, whereas enzymes had large CV. Large differences in CV were observed for major parameters among 7-8 test facilities. The changes in CV were grossly classified into 11. Our study revealed that a statistical significant difference is usually detected if there is a difference of 7% in mean values between the groups and the groups have a CV of about 7%. A parameter with a CV as high as 30% may be significantly different, if the difference of the mean between the groups is 30%. It would be ideal to use median value to assess the treatment-related effect, rather than mean, when the CV is very high. We recommend using CV of the body weight as a standard to judge the adverse effect level.

Key words: Coefficients of variation, Repeated-dose study, Quantitative value, Standard deviation, Chemical substance control law

INTRODUCTION

Repeated dose toxicity studies with rodents are usually conducted with a minimum of three treatment doses and a control (OECD, 1995). The quantitative data obtained from these studies are subjected to statistical analysis, using parametric or nonparametric statistical tools. If the data do not show heterogeneity and show a normal distribution, a parametric statistical tool is used, otherwise a nonparametric statistical tool. When the individual values of a parameter distribute in a wider range, it is most likely that the data show heterogeneity in variance. Distribution of data around mean can be estimated in terms of standard deviation and coefficient of variation (CV). CV is a numerical value where the proportion of the standard deviation in the mean value is shown as a percentage. Generally, the distribution of the quantitative values is broad for serum enzymes and narrow for electrolytes. Statistical significant difference of a parameter between groups is influenced by the difference of means between the groups, variance of the data and number of animals of the groups. A large difference observed between the mean values of control and dosage groups may not be statistically significant, if the variance of one or more groups explodes in a wider range.

Reports on the influence of CV in determining significant difference of quantitative values obtained from toxicity studies are rare. Matsuzawa *et al.* (1993) analyzed historical control data of clinical pathology testing provided by 67 member companies of the Japan Pharmaceutical Manufacturers Association covering study populations of approximately 14,000 rats, 10,000 dogs and 1,400 mon-

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keys. The authors assessed the potential factors contributing to variation in reference data based on weighted means and standard deviations. The authors described that the serum assay values showed greater variation than the plasma values.

In the present investigation, we examined the data of control groups of 28-day repeated-dose toxicity studies in rats performed according to the Chemical Substance Control Law (CSCL) in 12 test facilities. We examined 59 parameters of 153 studies. The number of animals per group, administration period and other factors were standardized according to the guidelines of CSCL. The distribution for each quantitative item (males and females separately) was converted into CV, and the influence of it in determining the significant difference between the groups was studied.

MATERIALS AND METHODS

The data investigated (Table 1) were from male and female SD rats of the control groups in the repeated-dose 28-day toxicity studies (Opening 153; MHLW, 2009) conducted by CSCL (NITE, 2007). CAS (Chemical Abstracts Service) numbers of the test substance administered in these studies are shown in Table 2. In these animals the vehicle was administered by oral gavage using a stomach tube. The number of animals in the group was 5-7 in most of the clinical examinations and organ weight determination and 10-12 in body weight and the feed consumption measurements. Most of the data were obtained on day 28 of the experimental period. However, few data pertaining to body weight, feed consumption, urinalyses and water consumption were obtained on days 29, 22-28, 26 and 24-26, respectively, during the experiment. The organ weight/body weight ratio was not included in the present investigation. CV (%) was calculated for each parameter using the standard formula.

The cluster analysis was conducted using the SAS JMP software (ver. 5.0; SAS Institute, Cary, NC, USA). The analysis of variance (ANOVA) and *t*-test were performed using software by Aoki (Aoki, 2010). Sex differences in CV were analyzed by the *F*-test for homogeneity of variance. When the variances were homogeneous at a significance level of 5%, Student's *t*-test was performed. When the variances were not homogeneous, Welch's *t*-test was performed. These *t*-tests were two-sided.

Items examined were (1) sex differences in CV of each quantitative value, (2) rank order of the parameters based on CV, (3) classification of CV of parameters by cluster analysis, (4) changes in CV of parameters in different test facilities, and (5) significant difference detection pattern when the difference between two groups was set constant and CV was changed.

RESULTS

CV for each quantitative item

Sex differences in CV of the 59 quantitative values are shown in Table 3. Statistically significant differences were observed in 12 items. Among them, large CVs were observed for prothrombin time (PT) and adrenal weights in the males compared with females (Table 4).

Rank order of parameters with regard to their CVs

CVs of the parameters of males and females were

Table 1. Parameters investigated

Item	Parameter
Animal care	Body weight (BW), Feed consumption (FC), Water consumption (WC)
Urinalyses	Urine volume (UV), Specific gravity (SG/urine), Osmotic pressure (OP/urine), *etc.*
Hematology	White Blood cell (WBC), Differential lymphocyte ratio (Lymph), Differential neutrophil ratio (Neut-seg), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet (PLT), Reticulocyte (RET), Fibrinogen (Fib), Prothrombin time (PT), Activated partial thromboplastin time (APTT), Methemoglobin, *etc.*
Blood chemistry	Total protein (TP), Albumin (Alb), Albumin/Globulin ratio (A/G), Total cholesterol (Cho), Total bilirubin (Bili), Triglyceride (TG), Glucose (Glu), Blood urea nitrogen (BUN), Creatinine (CRN), Triglyceride (TG), Phospholipid (PL), Alkaline phosphate (ALP), Lactic dehydrogenase (LDH), Cholinesterase (ChE), Creatine phosphokinase (CPK), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), γ Glutamyl transpeptidase (γ-GTP), Calcium (Ca), Inorganic phosphorus (IP), Sodium (Na), Potassium (K), Chloride (Cl), *etc.*
Absolute organ weights	Brain, Thymus, Thyroids, Heart, Lungs, Liver, Spleen, Kidneys, Adrenals, Testes, Epididymis, Ovaries, *etc.* The right and left attached table record adopts the right.

Table 2. CAS number of test substance administered in the 28-day repeated-dose toxicity studies

95-64-7	99-71-8	96-29-7	83-32-9	842-18-2	84-51-5	657-84-1	109-59-1	111-17-1
100-61-8	106-37-6	96-69-5	87-59-2	1066-40-6	88-18-6	824-78-2	109-64-8	112-26-5
103-69-5	121-47-1	111-41-1	95-33-0	16219-75-3	88-89-1	1328-53-6	1552-42-7	1025-15-6
105-99-7	526-78-3	119-47-1	97-52-9	25321-09-9	95-50-1	1333-16-0	3846-71-7	27676-62-6
88-53-9	1806-54-8	126-33-0	100-54-9	79-39-0	95-57-8	5039-78-1	4286-23-1	102-81-8
140-66-9	7756-94-7	526-73-8	100-69-6	80-09-1	96-76-4	6505-28-8	7803-57-8	620-92-8
538-75-0	26967-76-0	585-07-9	103-83-3	88-19-7	102-06-7	6731-36-8	12033-89-5	96-45-7
544-76-3	583-39-1	626-17-5	108-69-0	121-45-9	106-48-9	26471-62-5	26630-87-5	77-90-7
629-62-9	623-26-7	1570-64-5	123-30-8	793-24-8	108-39-4	76-83-5	56539-66-3	96-49-1
4390-04-9	1477-55-0	1843-05-6	1241-94-7	2416-94-6	109-70-6	98-51-1	80-51-3	100-47-0
5460-09-3	56-93-9	2216-69-5	3586-14-9	5707-44-8	110-30-5	108-73-6	95-32-9	107-95-9
86-87-3	87-02-5	3319-31-1	101-83-7	38640-62-9	123-07-9	5124-25-4	97-39-2	108-87-2
95-64-7	87-84-3	3648-21-3	127-68-4	51-28-5	591-27-5	6099-57-6	97-99-4	118-75-2
100-61-8	88-44-8	25154-52-3	130-13-2	75-59-2	599-64-4	79-27-6	99-94-5	121-60-8
103-69-5	95-63-6	78-51-3	135-51-3	80-43-3	620-17-7	101-72-4	100-74-3	134-62-3
141-02-6	141-17-3	461-72-3	517-23-7	1314-98-3	2580-78-1	3710-84-7	4435-53-4	5468-75-7
9014-90-8	9016-45-9	20679-58-7	25791-96-2	40766-31-2	112-18-5	29836-26-8	118-91-2	95-68-1

combined, arranged in order and are given in Table 5. The smallest CV was 0.74% for Na and next in order were Na, Cl, SG-urine, MCHC, Ca, MCV, MCH, HGB, brain weight, HCT, Alb, TP and RBC (up to 4% CV). The CV was between 4 and 10% for PT, lymph, K, lungs weight, Fib, testes weight, A/G ratio, IP, APTT, submaxillary gland weight, heart weight, kidneys weight, epididymis weight, PLT, liver weight and CRN. For Glu, BW, FC, adrenals weight, pituitary weight, BUN, Bili, spleen weight, ASAT, PL, ovaries weight, ALAT, thyroid weight, Cho, thymus weight and RET the CV was between 10-20%. For ALP, prostate gland weight, WC, ChE, WBC, OP-urine, LDH, uterus weight, methemoglobin and ã-GTP the CV varied between 20-30%, whereas for TG, UV, CPK and Neut-seg it varied between 30-40%.

The parameters with CV similar to body weight (7.09%) were PT, Fib, lungs weight, testes weight and submaxillary glands weight. Electrolytes showed smaller CVs. On the other hand, enzymes, urine volume, Neut-seg and methemoglobin showed larger CVs.

Classification of CV of quantitative values by cluster analysis

Mean, S.D., 95% confidence limits to mean (upper and lower), median, and maximum and minimum values for the 59 parameters of males and females were combined and cluster analysis was carried out using these parameters. The results of cluster analysis are shown in Table 6 and Fig. 1. There were 11 clusters. In cluster 1, 14 parameters were classified, whereas the number of parameters classified in clusters 2, 3, 4 and 5 were 12, 14, 6 and 7, respectively. In clusters 6-11, one parameter each having larger CV was classified.

Changes in CV of parameters with regard to the test facility

Several factors, like methodology employed, equipment used, expertise of personnel, laboratory environment, etc. to quantitatively determine a parameter in laboratory animals may differ from one test facility to the other. An analysis was carried out to understand the influence of the above factors on CVs of few selected parameters among the test facilities which carried out the 28 day repeated dose toxicity studies examined in the present investigation. The parameters selected and rationale for their selection is given in Table 7. Eight test facilities, viz., A1, B2, C3, D4, E5, F6, G7 and H8, which conducted 10 or more than 10 studies, were selected for the analysis. Differences among the facilities were analyzed by ANOVA. The parameter with minimum CV was considered as control. Comparison with control was done by a one-sided Student's or Welch's *t*-test. Test for homogeneity of variance (*F*-test) was conducted before the *t*-tests. Changes in CV of quantitative values according to the test facility are shown in Table 8.

Body weight: ANOVA did not indicate significant difference among the eight test facilities. However, the minimum CV value of F6 was significantly different from H8, E5, A1 and C3.

Feed consumption, Urine volume and Lymphocyte: ANOVA revealed a significant difference among the groups. The value of these parameters with the minimum

Table 3. Sex difference in CV (%) for each parameter

Parameter	Male					Female					Sex diff., *P*
	Mean ± S.D. (*N*)	95% confidence limit of mean	Median	Min.	Max.	Mean ± S.D. (*N*)	95% confidence limit of mean	Median	Min.	Max.	
BW	7.03 ± 1.77 (151)	6.75–7.32	6.91	3.22	12.8	7.15 ± 1.71 (151)	6.87–7.42	8.05	2.5	13.1	NS
FC	10.1 ± 2.90 (150)	9.66–10.5	10.2	1.56	20.0	12.3 ± 4.54 (150)	11.5–13.0	12.4	2.53	27.7	$P < 0.05$
WC	21.9 ± 9.18 (42)	15.0–24.7	20.1	8.33	39.6	24.6 ± 13.5 (42)	20.4–28.9	21.6	4.81	83.5	NS
UV	35.8 ± 13.7 (107)	33.2–38.4	34.6	10.8	81.8	39.5 ± 14.6 (109)	36.7–42.2	37.5	9.52	83.5	NS
SG-urine	1.45 ± 1.43 (82)	1.13–1.76	1.23	0.39	13.5	1.54 ± 1.12 (84)	1.29–1.78	1.43	0.45	10.4	NS
OP-urine	26.6 ± 9.01 (24)	22.8–30.4	25.8	8.87	46.3	26.5 ± 8.35 (24)	23.0–30.1	25.4	9.49	41.8	NS
HCT	3.48 ± 1.50 (150)	3.24–3.72	3.30	1.06	9.52	3.68 ± 1.51 (150)	3.44–3.93	3.42	0.64	9.35	NS
HGB	3.57 ± 2.37 (150)	3.19–3.96	3.24	0.64	20.8	3.46 ± 1.40 (150)	2.24–3.69	3.34	0.71	8.85	NS
RBC	4.16 ± 1.69 (150)	3.89–4.43	3.89	1.52	12.3	4.16 ± 1.48 (150)	3.92–4.40	4.05	1.1	8.98	NS
MCV	2.77 ± 1.13 (149)	2.59–2.95	2.73	0.44	6.66	2.55 ± 0.88 (149)	2.41–2.69	2.37	0.80	7.76	NS
MCH	2.89 ± 1.47 (149)	2.74–3.21	2.90	0.93	15.2	2.72 ± 0.94 (149)	2.57–2.87	2.61	0.50	5.39	NS
MCHC	1.62 ± 1.37 (149)	1.40–1.83	1.46	0.29	15.3	1.64 ± 1.13 (149)	1.46–1.82	1.44	0.29	10.2	NS
RET	17.7 ± 9.07 (119)	16.1–19.4	15.3	4.30	65.3	22.7 ± 11.4 (119)	20.6–24.7	20.8	1.22	72.2	$P < 0.05$
PLT	8.95 ± 3.58 (150)	8.38–9.53	8.31	1.94	28.3	9.64 ± 4.74 (150)	8.88–10.3	9.04	2.04	34.5	NS
WBC	24.6 ± 8.78 (150)	23.2–26.1	23.6	6.92	50.0	26.8 ± 8.04 (150)	25.5–28.1	26.4	6.38	50.0	$P < 0.05$
Neut-seg	39.8 ± 17.2 (150)	37.1–42.6	38.2	7.69	116	42.7 ± 17.1 (150)	39.9–45.4	41.5	0.00	95.0	NS
Lymph	4.88 ± 2.46 (150)	4.48–5.27	4.44	1.11	15.7	5.38 ± 3.17 (150)	4.87–5.89	4.76	1.07	22.5	NS
PT	8.63 ± 6.95 (150)	7.52–9.75	6.15	1.43	6.15	4.00 ± 2.20 (150)	3.65–4.36	3.55	0.57	16.7	$P < 0.05$
APTT	7.87 ± 3.07 (150)	7.38–8.36	7.40	2.51	17.6	7.51 ± 3.68 (150)	6.92–8.10	7.33	1.31	24.6	NS
Fib	6.40 ± 2.31 (37)	5.63–7.17	6.19	1.86	12.0	7.56 ± 2.94 (37)	6.58–8.55	7.32	2.27	13.6	NS
Methemoglobin	33.6 ± 20.2 (6)	12.3–54.9	34.3	12.7	56.0	34.9 ± 21.8 (6)	12.0–57.9	29.0	11.1	6.33	NS
BUN	13.3 ± 5.30 (150)	12.4–14.1	12.4	0.00	37.5	14.4 ± 6.25 (150)	13.4–15.4	13.3	4.54	13.7	NS
CRN	10.8 ± 6.26 (150)	9.83–11.8	10.6	0.00	10.6	10.9 ± 6.66 (150)	9.90–12.0	9.81	0.00	9.81	NS
Cho	16.8 ± 7.27 (151)	15.6–17.9	15.7	2.56	45.0	18.6 ± 7.55 (151)	17.4–19.8	18.0	6.67	53.3	$P < 0.05$
Bili	16.9 ± 20.8 (118)	13.3–20.6	11.7	0.00	100	16.0 ± 15.1 (118)	13.2–18.7	14.2	0.00	100	NS
TP	3.80 ± 1.98 (151)	3.48–4.12	3.69	0.00	20.7	4.26 ± 1.60 (151)	4.00–4.51	3.92	0.90	11.1	$P < 0.05$
Alb	3.70 ± 1.66 (151)	3.43–3.96	3.32	0.00	9.37	5.11 ± 2.40 (151)	4.72–5.49	5.06	0.00	13.5	$P < 0.05$
A/G	7.32 ± 3.19 (149)	6.81–7.83	7.29	1.06	19.1	8.26 ± 4.71 (149)	7.50–9.02	7.20	2.16	7.20	$P < 0.05$
Glu	10.2 ± 4.00 (150)	9.62–10.9	10.2	2.93	24.6	9.97 ± 3.62 (150)	9.39–10.5	10.1	2.58	18.2	NS
TG	34.8 ± 13.4 (151)	32.6–36.9	33.4	9.75	75.9	34.0 ± 17.1 (151)	31.3–36.7	32.3	7.40	96.4	NS
PL	12.7 ± 4.57 (25)	10.9–14.6	13.0	4.39	25.6	15.1 ± 5.95 (25)	12.6–17.6	15.2	6.61	26.3	NS
AST	13.4 ± 6.38 (151)	12.4–14.4	12.7	2.08	34.3	14.0 ± 6.52 (151)	12.9–15.0	13.1	4.34	42.1	NS
ALT	15.7 ± 6.65 (151)	14.6–16.7	15.3	3.57	40.7	16.8 ± 8.63 (151)	15.4–18.2	15.1	1.50	61.2	NS
ALP	18.8 ± 7.12 (150)	17.7–19.9	18.3	6.46	46.8	22.5 ± 7.31 (150)	21.3–23.6	22.2	6.68	45.1	$P < 0.05$
LDH	31.3 ± 15.8 (52)	26.9–35.7	26.1	10.3	78.4	27.7 ± 12.9 (52)	24.1–31.3	24.8	5.55	70.8	NS
γ-GTP	41.1 ± 48.2 (138)	33.1–49.2	27.9	0.00	210	51.4 ± 60.8 (142)	41.4–61.4	33.1	0.00	318	NS
CPK	48.3 (2)					36.3 (2)					
ChE	21.9 ± 11.3 (27)	17.4–26.3	22.2	5.50	51.8	26.4 ± 11.7 (27)	21.8–31.1	24.6	9.16	57.5	NS
Na	0.73 ± 0.30 (149)	0.68–0.77	0.69	0.06	1.99	0.76 ± 0.32 (149)	0.71–0.81	0.70	0.13	1.70	NS
K	6.06 ± 4.99 (149)	5.25–6.86	5.02	1.03	41.0	6.47 ± 5.74 (149)	5.55–7.39	5.57	0.00	50.6	NS
Cl	1.34 ± 0.60 (149)	1.24–1.44	1.21	0.37	4.68	1.30 ± 0.65 (149)	1.20–1.40	1.19	0.37	6.03	NS
Ca	2.70 ± 1.14 (149)	2.51–2.88	2.38	0.61	8.79	2.73 ± 1.12 (149)	2.55–2.91	2.47	0.86	7.77	NS
IP	6.33 ± 3.08 (149)	5.83–6.82	5.66	1.16	22.7	9.24 ± 3.56 (149)	8.67–9.81	8.82	1.36	23.7	$P < 0.05$
Brain weight	3.41 ± 1.40 (150)	3.24–3.69	3.33	0.47	9.26	3.54 ± 1.25 (150)	3.34–3.74	3.35	0.82	7.21	NS
Submaxillary gland weight	9.31 ± 2.39 (3)	3.36–15.2	10.7	6.55	10.7	5.83 ± 2.71 (3)	0.00–12.5	7.31	2.7	7.5	NS
Pituitary weight	12.0 ± 3.92 (42)	10.8–13.2	11.9	4.2	20.6	13.8 ± 4.27 (42)	12.5–15.1	12.4	6.20	24.9	$P < 0.05$
Thyroid weight	16.6 ± 5.14 (51)	15.2–18.1	16.1	5.00	28.4	15.8 ± 5.08 (51)	14.4–17.2	15.9	3.37	15.9	NS
Thymus weight	18.2 ± 5.95 (124)	17.2–19.3	17.2	5.79	46.4	18.1 ± 6.68 (124)	16.9–19.2	17.7	4.72	49.3	NS
Heart weight	8.25 ± 3.43 (108)	7.60–8.90	7.62	2.83	20.6	8.85 ± 4.41 (108)	9.02–9.68	8.17	3.03	8.17	NS
Lungs weight	7.16 ± 4.28 (49)	5.92–8.39	6.65	1.45	31.6	7.16 ± 3.43 (49)	6.17–8.14	6.66	0.99	23.2	NS
Liver weight	9.95 ± 3.53 (150)	9.39–10.5	9.63	2.46	19.4	9.19 ± 3.26 (150)	8.67–9.71	8.75	2.63	18.4	NS
Kidneys weight	8.51 ± 3.69 (150)	7.92–9.10	8.11	1.55	32.4	7.92 ± 2.59 (150)	7.50–8.33	7.73	1.91	15.6	NS
Spleen weight	13.9 ± 5.80 (133)	12.9–14.1	13.0	2.73	33.3	13.3 ± 4.89 (150)	12.4–14.1	12.7	0.16	28.9	NS
Adrenals weight	13.1 ± 4.75 (150)	12.3–13.8	12.9	3.12	24.7	11.8 ± 4.81 (150)	11.0–12.6	11.5	2.66	27.2	$P < 0.05$
Testes v	7.44 ± 4.16 (150)	6.77–8.10	6.93	1.43	35.4						
Epididymis weight	8.88 ± 4.33 (88)	7.96–9.80	8.17	1.88	28.2						
Prostate weight	8.88 ± 4.33 (88)	7.96–9.80	8.17	1.88	28.2						
Ovaries weight						14.7 ± 4.93 (145)	13.9–15.5	14.4	3.40	25.7	
Uterus weight						27.6 ± 13.7 (16)	20.3–34.9	26.3	6.21	55.5	

NS, not significant difference. Vide Table 1 for abbreviations.

Table 4. Parameters that showed changes in CV with regard to sex

Sex	Parameter that increased compared to the other sex
Male	PT, Adrenals
Female	FC, RET, WBC, Cho, TP, Alb, A/G, ALP, IP, Pituitary

CVs were different from those respective parameters of 7 test facilities.

GOT and Sodium: Differences were observed among the test facilities. Compared to the value of G7, GOT was different in the other test facilities, whereas sodium was different in 5 facilities compared to the value of C3.

Brain, liver and spleen weights: These parameters were not statistically different among the facilities as per ANOVA. However, the value with smallest CV was significantly different in 6 facilities in the case of brain weight, 4 facilities in the case of liver weight and 3 facilities in the case of spleen weight as per t test.

Rank order of test facilities with regard to the parameters evaluated is given in Table 9. The mean CV ranks in the increasing order were D4, F6, A1, H8, G7, C3, E5, and B2.

Next, a cluster analysis was performed using the data given in Table 9 to understand the features of each test facility. Test facility G7 was excluded because data on urine volume were not available in this test facility.

The clusters were grossly divided into two bunches (Fig. 2). One bunch consisted of D4 and F6, with several parameters having small CV. The second bunch included five test facilities that had two sub-bunches, A1, H8, and E5 in the first bunch and C3 and B2 in the second bunch. The distance (difference) between D4 and B2 was the largest.

Significant difference detection pattern when the difference of mean between two groups is set constant and the CV changed

Changes in body weight are considered as the most important index in toxicity studies (MHLW, 2009). In the present study, the power of detection pattern of statistically significant difference as result of change in CV was assessed using the body weight values. In a study (CAS No. 26471-62-5), the value of 10 males in the control group was 336 g (100%) at 4 weeks after the test substance administration. A normal distribution was assumed for these rats with a CV of 7.7%. The mean value of the dosage group was set as constant (312 g, 92.8%). The CV of both the groups was changed in the range of 2 to 45%. These data were analyzed by a two-sided Student's *t*-test.

A significant difference ($P < 0.05$) was observed if the

Table 5. Rank order of parameters with regard to CV (%)

Rank order	Parameter	Mean ± S.D. (Number of study*)	95% confidence limit in mean	Median
1	Na	0.74 ± 0.31 (298)	0.71–0.78	0.69
2	Cl	1.32 ± 0.63 (298)	1.25–1.39	1.25
3	SG/urine	1.49 ± 1.28 (166)	1.30–1.69	1.33
4	MCHC	1.63 ± 1.26 (298)	1.49–1.77	1.44
5	MCV	2.66 ± 1.02 (298)	2.55–2.78	2.54
6	Ca	2.72 ± 1.13 (298)	2.59–2.84	2.46
7	MCH	2.85 ± 1.24 (298)	2.71–2.99	2.87
8	Brain	3.51 ± 1.32 (300)	3.36–3.66	3.35
9	HGB	3.52 ± 1.94 (300)	3.30–3.74	3.27
10	HCT	3.58 ± 1.51 (300)	3.41–3.75	3.37
11	TP	4.03 ± 1.82 (302)	3.82–4.23	3.85
12	RBC	4.16 ± 1.59 (300)	3.98–4.34	3.97
13	Alb	4.40 ± 2.18 (302)	4.16–4.65	3.73
14	Lymph	5.13 ± 2.84 (300)	4.81–5.45	4.55
15	K	6.26 ± 5.38 (298)	5.65–6.87	5.48
16	PT	6.32 ± 5.64 (300)	5.68–6.96	4.27
17	Fib	6.98 ± 2.69 (74)	6.36–7.61	6.88
18	Lungs	7.16 ± 3.86 (98)	6.38–7.93	6.65
19	BW	7.09 ± 1.74 (302)	10.7–11.6	10.7
20	Testes	7.44 ± 4.16 (150)	6.77–8.10	6.93
21	Submaxillary gland	7.57 ± 2.98 (6)	4.48–10.7	7.40
22	APTT	7.69 ± 3.39 (300)	7.31–8.07	7.33
23	IP	7.78 ± 3.63 (298)	7.37–8.20	7.31
24	A/G	7.79 ± 4.05 (298)	7.33–8.25	7.24
25	Kidneys	8.22 ± 3.20 (300)	7.85–8.58	7.89
26	Heart	8.55 ± 3.95 (216)	8.02–9.08	7.87
27	Epididymis	8.88 ± 4.33 (88)	7.96–9.80	8.17
28	PLT	9.28 ± 4.21 (300)	8.82–9.77	8.70
29	Liver	9.57 ± 3.41 (300)	9.18–9.96	9.19
30	Glu	10.1 ± 3.81 (300)	9.69–10.5	10.2
31	CRN	10.9 ± 6.45 (300)	10.1–11.6	10.0
32	FC	11.2 ± 3.96 (300)	10.7–11.6	10.7
33	Adrenals	12.4 ± 4.81 (300)	11.9–13.0	12.1
34	Pituitary	12.9 ± 4.17 (84)	12.0–13.8	12.2
35	Spleen	13.6 ± 5.36 (266)	12.9–14.2	12.8
36	ASAT	13.7 ± 6.44 (302)	13.0–14.4	12.8
37	BUN	13.8 ± 5.81 (300)	13.2–14.5	12.6
38	PL	13.9 ± 5.38 (50)	12.4–15.4	13.3
39	Ovaries	14.7 ± 4.90 (145)	13.9–15.5	14.4
40	Thyroid	16.2 ± 5.10 (102)	15.2–17.2	16.1
41	ALAT	16.2 ± 7.71 (302)	15.4–17.1	15.2
42	Bili	16.4 ± 17.8 (236)	14.2–18.7	12.6
43	Cho	17.7 ± 7.46 (302)	16.8–18.5	16.8
44	Thymus	18.1 ± 6.31 (248)	17.4–18.9	17.6
45	Prostate	18.9 ± 9.74 (7)	9.92–27.9	20.5
46	RET	20.2 ± 10.5 (238)	18.9–21.5	18.4
47	ALP	20.6 ± 7.43 (300)	19.8–21.5	20.2
48	WC	23.3 ± 11.5 (84)	20.7–25.8	21.5
49	ChE	24.2 ± 11.6 (54)	21.0–27.3	23.0
50	WBC	25.7 ± 8.47 (300)	21.8–26.7	25.1
51	OP/urine	26.6 ± 8.59 (48)	24.1–29.1	25.5
52	Uterus	27.6 ± 13.7 (16)	20.3–34.9	26.3
53	LDH	29.5 ± 14.5 (104)	36.7–32.3	25.8
54	Methemoglobin	34.3 ± 20.1 (12)	21.5–47.1	29.0
55	TG	34.4 ± 15.4 (302)	32.7–36.1	33.0
56	UV	37.9 ± 14.2 (216)	35.7–39.6	35.3
57	Neut-seg	41.3 ± 17.1 (300)	39.3–43.2	40.0
58	CPK	42.3 ± 16.3 (4)	16.3–68.2	36.3
59	γ-GTP	46.3 ± 55.1 (280)	39.9–52.8	30.0

* Values given in parentheses are number of observations from both males and females.

difference in body weight mean was about 7% between the groups and the CV was 7-8% (Table 10). A significant difference was observed for the γ-GTP, CPK, neutrophil cell, urine volume, and triglyceride levels which showed a CV of 30% or more and a 30% difference of mean between groups.

Table 6. Classification of CVs of 59 parameters by cluster analysis

Custer No.	Parameter
1	BW, Fib, Submaxillary gland weight, PLT, Epididymis weight, Kidneys weight, Heart weight, APTT, IP, A/G ratio, Lungs weight, Testes weight, PT, K
2	FC, Adrenals weight, Gul, Liver weight, BUN, Spleen weight, ASAT, CRA, PL, Pituitary weight, Thyroid weight, Ovaries weight
3	SG-urine, MCHC, Na, Cl, HCT, Brain weight, RBC, MCV, Ca, MCH, HGB, TP, Alb, Lymph
4	WC, ChE, LDH, Uterus weight, OP-urine, WBC
5	RET, Cho, ALAT, ALP, Thymus weight, Prostate gland weight
6	Bili
7	UV, TG
8	Methemoglobin
9	Neut-seg
10	CPK
11	γ-GTP

DISCUSSION

We calculated CV for 59 quantitative values obtained from the 153 numbers of 28-day repeated-dose toxicity studies carried out in accordance with the CSCL in 12 test facilities. We conclude the following from our findings:

1. Sex difference in CV was observed in 12 out of 59 items including body weight. Larger CV was observed in 10 items of male.

2. The quantitative value with the smallest CV was 0.74% for sodium. Electrolytes and calculated hematology values showed smaller CVs, whereas, enzymes showed larger CVs.

3. The values based on their CVs were classified into 11 clusters, which were grossly divided into 4 to 5 bunches.

4. CVs for liver, brain and spleen weights were similar in all the test facilities studied. However, large differences in body weight and some clinical laboratory tests were observed in few test facilities. Therefore it is most likely that statistically significant differences could be detected for the data with small CVs in some test facilities. Similarly, no statistical significant difference will be shown by the data with large CVs in some test facilities.

5. A significant difference is seen at 5% probability level, if the difference of the mean value between the groups is about 7% and CV 7%-8%.

6. A statistically significant difference can be detect-

Table 7. Influence of laboratory factors on CVs of parameters analysed. List of parameters selected and the rationale for their selection

Parameter	Rationale for selection
Body weight	Toxicity of a chemical is primarily reflected on body weight. Several factors like poor animal husbandry, animal house condition and improper handling of the animals can affect body weight.
Feed consumption	The factors that may affect body weight can affect feed consumption of the animals. Accurate measurement of feed consumption is not an easy task because of spillage. Feed hoppers vary from one laboratory to the other.
Urine volume	Metabolic cages are used for urine collection. These cages differ from one testing facility to the other.
Lymphocyte	Lymphocyte count usually done under a microscope, varies among the technicians who perform the count.
GOT	Variation in GOT estimation may occur with regard to the analytical reagents, methodology, instrument calibration and the nature of the plasma/serum sample (hemolysed samples may show erratic GOT values).
Sodium	Fluctuation in sodium is the least compared to other clinical chemistry parameters.
Brain weight	Absolute weight of the brain can be determined accurately in all the test facilities
Liver weight	Onslaught of toxicity effect of a chemical is reflected on liver and variation in absolute weight of this organ is usually seen.
Spleen weight	Most of the test facilities determine absolute weight of the spleen accurately.
Other parameters	Instruments used to determine other hematology and blood chemistry parameters are not different from those used to determine above parameters. Enzymes that show very larger variations are not also not included. Erythrocyte indices *etc.* have a lot of calculation values. However, these parameters were seemed no large changes.

Table 8. Changes in CV of quantitative values according to test facilities

Parameter	Test facility	Mean ± S.D. (%)	No. of study (male + female)	*P* ANOVA	*P* *t*-test
Body weight	F6	6.36 ± 1.81 (100)	20	0.485	vs. F6
	D4	6.81 ± 1.54 (107)	40		0.156
	G7	6.98 ± 1.45 (109)	36		0.084
	B2	7.08 ± 1.83 (111)	28		0.091
	H8	7.17 ± 1.50 (112)	30		0.045
	E5	7.18 ± 1.27 (112)	26		0.038
	A1	7.31 ± 1.87 (114)	48		0.028
	C3	7.32 ± 2.14 (115)	50		0.040
Feed consumption	D4	7.00 ± 2.11 (100)	40	0.000	vs. D4
	B2	10.6 ± 4.03 (152)	28		< 0.01
	A1	10.7 ± 3.04 (153)	48		< 0.01
	G7	11.4 ± 3.25 (163)	34		< 0.01
	C3	12.2 ± 3.55 (174)	49		< 0.01
	H8	12.6 ± 3.74 (180)	30		< 0.01
	E5	12.8 ± 4.92 (183)	26		< 0.01
	F6	13.2 ± 4.16 (189)	20		< 0.01
Urine volume	F6	1.43 ± 0.40 (100)	20	0.000	vs. F6
	D4	19.3 ± 17.2 (1349)	14		< 0.01
	C3	29.0 ± 10.8 (2027)	36		< 0.01
	H8	35.5 ± 12.0 (2482)	28		< 0.01
	A1	35.9 ± 15.6 (2510)	46		< 0.01
	E5	42.4 ± 16.5 (2965)	26		< 0.01
	B2	44.4 ± 13.8 (3104)	28		< 0.01
Lymphocyte	E5	3.10 ± 0.99 (100)	26	0.000	vs. E5
	H8	4.32 ± 1.97 (139)	28		< 0.01
	A1	4.35 ± 2.65 (140)	48		< 0.01
	D4	4.44 ± 1.72 (143)	40		< 0.01
	H8	5.49 ± 1.94 (177)	36		< 0.01
	C3	6.16 ± 3.52 (198)	50		< 0.01
	B2	6.49 ± 3.05 (209)	28		< 0.01
	F6	7.38 ± 4.04 (238)	20		< 0.01
GOT	G7	10.1 ± 4.36 (100)	36	0.001	vs. G7
	H8	12.8 ± 5.78 (126)	30		0.022
	C3	12.9 ± 8.29 (127)	50		0.026
	A1	12.9 ± 6.39 (127)	48		0.012
	F6	14.9 ± 4.95 (147)	20		< 0.01
(Continued)					
	D4	15.3 ± 4.25 (151)	40		< 0.01
	E5	15.8 ± 7.08 (156)	26		< 0.01
	B2	16.0 ± 6.41 (158)	28		< 0.01
Sodium	C3	0.61 ± 0.26 (100)	50	0.000	vs. C3
	F6	0.62 ± 0.21 (101)	20		0.452
	D4	0.65 ± 0.19 (106)	40		0.184
	A1	0.72 ± 0.29 (118)	48		0.023
	B2	0.79 ± 0.25 (129)	28		< 0.01
	H8	0.81 ± 0.28 (132)	30		< 0.01
	E5	0.82 ± 0.43 (134)	26		0.015
	G7	0.89 ± 0.29 (145)	34		< 0.01
Brain weight	F6	2.79 ± 0.87 (100)	20	0.081	vs. F6
	D4	3.22 ± 1.12 (115)	38		0.074
	A1	3.40 ± 1.20 (121)	48		0.022
	H8	3.45 ± 1.09 (123)	30		0.014
	G7	3.56 ± 1.49 (127)	36		< 0.01
	E5	3.58 ± 1.17 (128)	26		< 0.01
	C3	3.79 ± 1.33 (135)	50		< 0.01
	B2	3.83 ± 1.72 (137)	28		< 0.01
Liver weight	F6	8.17 ± 2.41 (100)	20	0.726	vs. F6
	D4	9.35 ± 3.67 (115)	38		0.102
	A1	9.46 ± 3.88 (121)	48		0.052
	H8	9.53 ± 2.41 (123)	30		0.028
	E5	9.61 ± 3.31 (127)	26		0.054
	B2	9.69 ± 3.19 (128)	28		0.040
	G7	9.70 ± 2.74 (135)	36		0.021
	C3	10.0 ± 4.05 (137)	50		0.010
Spleen weight	F6	11.4 ± 4.49 (100)	20	0.549	vs. F6
	D4	12.6 ± 3.60 (110)	30		0.212
	A1	13.1 ± 5.63 (114)	48		0.129
	G7	13.6 ± 5.12 (119)	28		0.072
	E5	13.9 ± 6.06 (121)	20		0.081
	H8	13.9 ± 4.47 (121)	30		0.032
	B2	14.1 ± 5.44 (123)	28		0.042
	C3	14.3 ± 6.40 (125)	38		0.042

Table 9. Rank order of test facilities with regard to the parameters evaluated. Ranking is done from smallest to largest CV

Parameter	Test facility							
	D4	F6	A1	H8	G7	C3	E5	B2
Body weight	2	1	7	5	3	8	6	4
Feed consumption	1	8	3	6	4	5	7	2
Urine volume	2	1	5	4		3	6	7
Lymphocyte	4	8	3	2	5	6	1	7
GOT	6	5	4	2	1	3	7	8
Sodium	3	2	4	6	8	1	7	5
Brain weight	2	1	3	4	5	7	6	8
Liver weight	2	1	3	4	7	8	5	6
Spleen weight	2	1	3	6	4	8	5	7
Mean rank	2.6	3.1	3.8	4.3	4.6	5.4	6.6	7.1

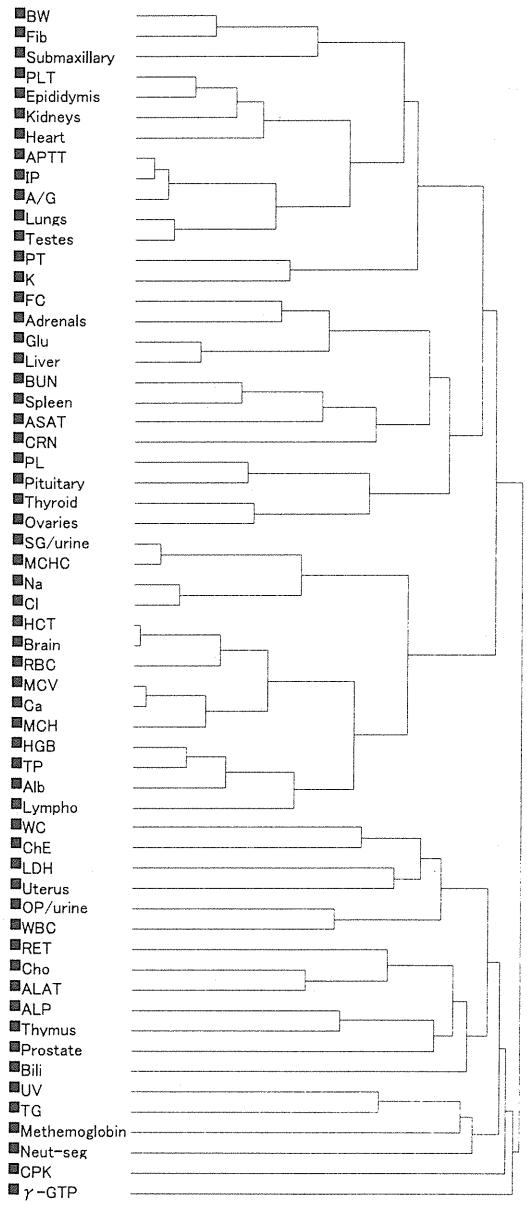

Fig. 1. Classification of CV of quantitative value by cluster analysis.

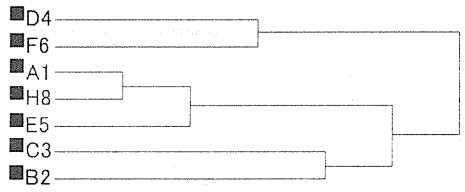

Fig. 2. Classification of CV of test facilities by cluster analysis.

Table 10. Significant difference power pattern when the body weight difference between two groups is set to a constant and CV is changed

Control group: 336 g vs. dosed group: 312 g	
Coefficient of variation (%) of two groups	*P* value
2	0.000
3	0.000
4	0.000
5	0.003
6	0.012
7	0.029
8	0.053
9	0.082
10	0.115
12	0.184
14	0.252
16	0.314
18	0.369
20	0.418
25	0.516
30	0.587
35	0.641
40	0.683
45	0.717

ed for parameters with extremely small CVs even for a smaller difference in mean values.

Information on the influence of CV in determining significant difference of quantitative parameters obtained from animal toxicity studies is scarce. Aoyama (2005) suggested that when the number of animals is adjusted, the decentralization of data, like body weight and the organ weight, becomes comparatively smaller, and a CV of about 10% is obtained. CV for blood levels of various hormones, even data in the control group are large. Often, the standard deviation exceeds the mean value by more than 50% for these parameters. Present study also reveals similar findings. CVs have greater influence in determining the significant difference of a parameter in repeated dose toxicity studies and the CVs vary considerably for certain parameters in different test facilities. Thus priority should be given to a judgment of toxicological effects, not to a statistically significant difference when such a dif-

ference is smaller than that based on CV of body weight measurements.

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REFERENCES

AOKI (2010): http://aoki2.si.gunma-u.ac.jp/JavaScript/oneway-ANOVA2.html

Aoyama, H. (2005): Applications and limitations of *in vivo* bioassays for detecting endocrine disrupting effects of chemicals on mammalian species of animals. J. Natl. Inst. Public Health, **54**, 29-34.

Matsuzawa, T., Nomura, M. and Unno, T. (1993): Clinical pathology reference ranges of laboratory animals. J. Vet. Med. Sci., **55**, 351-362.

MHLW (2009): Ministry of Health, Labour and Welfare: http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPage.jsp

NITE (2007): National Institute of Technology and Evaluation. http://www.safe.nite.go.jp/kasinn/pdf/28test.pdf

OECD. Organization for Economic Cooperation and Development (1995): OECD Guidelines for Testing of Chemicals. Repeated Dose 28-Day Oral Toxicity Study in Rodents. No. 407, pp. 8.