Fundamental Toxicological Sciences (Fund. Toxicol. Sci.)
Vol.1, No.1, 1-6, 2014

Letter

Investigation of organ-specific assessment factors related to sub-acute and sub-chronic toxicity studies

Jun-ichi Takeshita[1], Atsuo Kishimoto[2] and Masashi Gamo[1]

[1]Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan
[2]Graduate School of Public Policy, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

(Received July 4, 2014; Accepted July 16, 2014)

ABSTRACT — In risk assessment of chemicals, we often use default assessment factors to compensate for lack of knowledge. Such assessment factors can be especially useful for regulatory decisions. The present study focuses on assessment factors related to exposure duration, especially under sub-acute and sub-chronic conditions; and discussions attempt to utilize chemical-specific toxicological data. Most previous studies have not focused on target organs, but recent reports such as Malkiewicz *et al.* (2009) suggest that assessment factors may be target organ-dependent. Therefore, we addressed selected target organs (liver, kidney, blood, and body weight) by investigating assessment factors for these target organs. Using existing data, we calculated the ratio of the no-observed-effect level (NOEL) derived from sub-acute studies to the NOEL derived from sub-chronic studies, to assess for effects involving individual target organs (liver, kidney, blood, or body weight). Then, we compared these ratios with the ratio derived from the substances' sub-acute and sub-chronic NOELs (the minimum values among all four target organs' NOELs) by using the Dunnett's multiple comparison test. Our analysis indicates that effects involving liver, kidney, and body weight need not be treated independently, although the effect on blood should be treated separately. Based on our results, we discuss potential refinement of assessment factors to reflect exposure duration.

Key words: Risk assessment, Assessment factor, Exposure duration, Sub-acute toxicity study, Sub-chronic toxicity study, Chemical-specific factor

INTRODUCTION

In risk assessment of chemicals, we often use default assessment factors to compensate for lack of knowledge. Such assessment factors can be especially useful for regulatory decisions, such as establishing acceptable daily intakes (ADIs). According to the early guidelines used by the United States Food and Drug Administration (US FDA), a 100-fold safety factor should be used for food additives (Lehman and Fitzhugh, 1954). This value was among the earliest applications of quantitative assessment factors. The US Environmental Protection Agency (US EPA) subsequently established default uncertainty factors of 10-fold for inter-species extrapolation, inter-individual variations, exposure duration, etc. (US EPA, 1988).

Renwick (1991, 1993) proposed to divide the uncertainty factors related to inter-species extrapolation and inter-individual variations into two sub-factors, toxicokinetics (TK) and toxicodynamics (TD). The World Health Organization / International Program on Chemical Safety (WHO/IPCS, 1994, 1999) subsequently adopted this idea, suggesting values of 4 and 2.5 for TK and TD, respectively, for inter-species extrapolation, and 3.16 and 3.16 for TK and TD, respectively, for inter-individual variations. Also, WHO/IPCS (2005) proposed the use of chemical-specific toxicological data such as actual data on TK and/or TD instead of default assessment factors if possible. On the other hand (and as surveyed below), several studies employed probabilistic distributions, instead of deterministic assessment factors, basing their proposal on analysis of existing toxicological data.

The present study focuses on assessment factors related to exposure duration, especially sub-acute (SA; majority of the studies: 28 days) and sub-chronic (SC; majority

Correspondence: Jun-ichi Takeshita (E-mail: jun-takeshita@aist.go.jp)

of the studies: 90 days); and discusses utilizing chemical-specific toxicological data to establish these assessment factors. In fact, the Japanese Law Concerning the Evaluation of Chemical Substances and Regulation of their Manufacture, etc., mandates SA studies; and applications for registration of agricultural chemicals require SC studies in Japan. Further, the Registration, Evaluation, and Authorization of Chemicals (REACH) regulation requires either SA or SC studies. Therefore, determining the target organ's effects of different substances sometimes will require comparing data obtained from distinct (SA vs. SC) assay types.

Here we survey the literature pertaining to the present study. US EPA (2002), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2003), and European Chemicals Agency (ECHA, 2012) each have recommended distinct assessment factors related to exposure duration. US EPA (2002) proposed a factor of 10 for extrapolating from sub-chronic to chronic studies. ECETOC (2003) and ECHA (2012) recommended the same factors as each other, proposing values of 2 and 6 for extrapolating from sub-chronic and sub-acute, respectively, to chronic studies, and a value of 3 for extrapolating from sub-acute to sub-chronic. Also, several previous research articles that focused on exposure duration adopted a probabilistic distribution analysis. Most of these previous studies calculated and analyzed (from existing data) the ratio of the no-observed-(adverse-)effect level (NO(A)EL) of sub-acute studies (NO(A)EL_SA) to the NO(A)EL of sub-chronic studies (NO(A)EL_SC). Kalberlah and Schneider (1998), Kalberlah *et al.* (2002), and Schneider *et al.* (2005) concluded that the geometric mean (GM) of the ratio of NO(A)EL_SA to NO(A)EL_SC was (respectively) 2-3, 3.3, or 3.95. Groeneveld *et al.* (2004) described a data method for analyzing existing oral experimental data from rats and mice. These researchers concluded that the GM was 1.63 in the case of rats alone, and 1.60 in the case of both rats and mice; with geometric standard deviations (GSDs) of 3.53 and 3.29, respectively (Groeneveld *et al.*, 2004). However, that analysis employed data from only three substances in the case of mice. Based on experimental results for 82 substances, Woutersen *et al.* (1984) concluded that, for 95% of the target substances, the value of NO(A)EL_SC was greater than or equal to the NO(A)EL_SA value divided by 10. On the other hand, Malkiewicz *et al.* (2009) classified the same data as Kalberlah and Schneider (1998) into mortality and non-lethal endpoints, permitting the calculation of NOAEL ratios (that is, NOAEL_SA/NOAEL_SC) for mortality/mortality, mortality/non-lethal endpoints, and non-lethal endpoints/non-lethal endpoints. These researchers obtained respective GMs (GSDs) of 1.4 (1.4), 4.4 (1.9), and 2.7 (2.4), respectively, and indicated that the NOAEL ratios depended on the respective endpoints (Malkiewicz *et al.*, 2009).

Most previous studies have not focused on target organs; however, Malkiewicz's study (Malkiewicz *et al.*, 2009) suggested that assessment factors may be target organ-dependent. Therefore, the present study focused on selected internal organs (liver, kidney, blood, and body weight) and corresponding assessment factors. More precisely, for a given substance, we first set both NOEL_SA (mg/kg/day) and NOEL_SC (mg/kg/day) for internal organs (and body weight); as the substance's NOEL_SA and NOEL_SC, we set the minimum values of NOEL_SA and NOEL_SC among the four target organ's NOEL_SA and NOEL_SC, respectively. Hereafter, we say them NOEL_SA and NOEL_SC from the combined data, respectively. Then, we calculated the ratio of NOEL_SA to NOEL_SC for effects involving the liver, kidney, blood, and body weight, and compared these ratios with the ratios derived from the combined data. In other words, we determined organ-specific assessment factors for each internal organ (and body weight). Such values would be used when comparing a target organ's effect, that is, the effects involving each internal organ (and body weight) during sub-acute and sub-chronic studies among different substances. Moreover, this analytical approach was expected to provide a chemical-specific result, permitting refinement of assessment factors related to exposure duration: if there are assessment factors for each target organ, then for a given chemical substance, we should be able to apply an assessment factor depending on the target organ. Given a substance's NOEL_SA, such an assessment factor should permit extrapolation from sub-acute to sub-chronic (or chronic) conditions. Namely, we were able to consider a given substance's properties, which was the target organ determining the substance's NO(A)EL in the study.

The paper is organized as follows. Section 2 outlines the data and statistical methods employed in the present study. Section 3 summarizes the major results of the analysis. Section 4 discusses the results and summarizes the study.

MATERIALS AND METHODS

Data collection

We assembled toxicity data from the Initial Risk Assessment Report in the Chemical Risk Assessment and Development of Risk Assessment Methods in the Program for Comprehensive Assessment and Management of

Chemicals (FY2001-2006), one of the research achievements of the Chemicals Evaluation and Research Institute (CERI) and the National Institute of Technology and Evaluation (NITE) of the New Energy and Industrial Technology Development Organization (NEDO) project. The reports can be accessed on the NITE website (NITE, 2010).

The Initial Risk Assessment Report includes results for 167 substances, all of which are listed in the Japan Pollutant Release and Transfer Register (PRTR) as Class 1 Designated Chemical Substances. The object substances are 354 chemicals with high emission rate and hazardousness. The toxicity data on the 167 Class 1 substances has been verified by an advisory body on chemical substances, a body convened by the Ministry of Economy, Trade and Industry (METI). We therefore considered these substances, and the related toxicity data, to have sufficient representativeness to be used in our analysis.

In the present study, we used data from experiments evaluating changes in the liver, kidney, blood, and body weight. The reasons for focusing on these four kinds of effects in the present study were as follows. The liver, blood, and kidney are the top three target organs for determining the NO(A)EL for a given chemical substance (e.g., Yamada *et al.*, 2013); and body weight is a typical observation item in repeated-dose studies. As in previous studies, we used data from oral exposure experiments; since related data on mice was limited, we focused exclusively on data from experiments on rats.

With reference to the classification in Groeneveld *et al.* (2004), we established two categories of exposure duration in the oral repeated-dose toxicity studies, as follows: 21-42 days for SA studies (majority of the studies: 28 days), and 49-183 days for SC studies (majority of the studies: 90 days). When, for a given substance, there was more than one study examining the same exposure duration category on the same target organ, the study with the geometric mean NOEL was selected for additional analysis. Further, for a given substance, we denoted the NOEL for the four combined (the substance's NOEL) by the minimum value among the NOELs of the four target organs.

We calculated the NOEL_SA to NOEL_SC ratio for each target organ and, for the four combined. We here note that some studies pointed out that the use of distributions of ratios obtained from benchmark doses preferred in comparison to use of the ratios of NO(A)ELs in recent years (Bokkers and Slob, 2005). Nonetheless, we used NO(A)ELs with reference to the previous studies such as Malkiewicz *et al.* (2009) and Groneneveld *et al.* (2004).

Data analysis

All statistical analyses were performed using Excel 2011 (Microsoft, Redmond, WA, USA).

The ratios of NOEL_SA to NOEL_SC were statistically analyzed by determining the geometric mean (GM), representing a central estimation of the ratio, and the geometric standard deviation (GSD), as a measure of the variation in the distribution.

The ratios were analyzed for statistically significant differences between the effects involving the respective target organs and the combined data, using the Dunnett's multiple comparison test at the 5% level of significance.

RESULTS

In Fig. 1, each substance's ratio of NOEL_SA to NOEL_SC is presented for effects involving the liver, kidney, blood, and body weight, and the combined data. Table 1 shows the number of substances for which ratios were calculated, and the GM and GSD of the distributions, for effects on the four target organs, this is, for liver, kidney, blood, and body weight, and for the combined data.

The Dunnett's test, used to verify the homogeneity of means, indicated a statistically significant difference between the GM of the ratios for effects involving the blood and the GM of the ratio for the combined data. On the other hand, there were no statistically significant

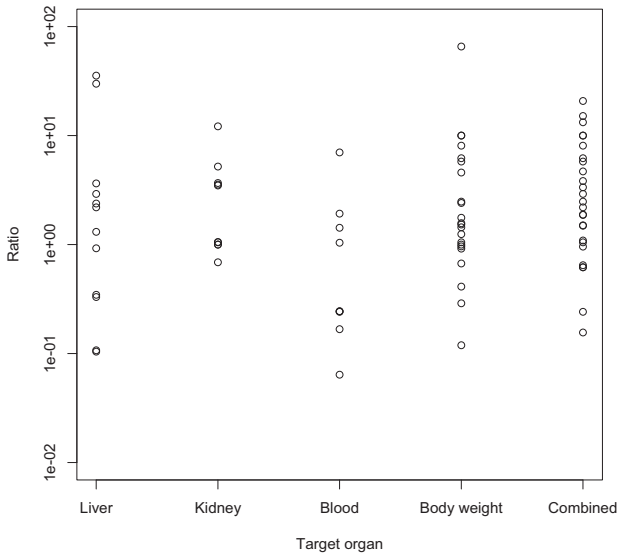

Fig. 1. Each substance's ratio of NOEL_SA to NOEL_SC for effects involving the liver, kidney, blood, or body weight, or the combined data.

Table 1 Exposure duration parameters for effects involving the liver, kidney, blood, or body weight, and for the combined data, derived from NOEL ratios obtained from the study database.

	Combined	Liver	Kidney	Blood	Body weight
n	26	13	10	9	22
GM	2.4	1.5	2.2	0.53	2.0
GSD	3.5	6.1	2.5	4.0	3.9

n = number of ratios, GM = geometric mean, GSD = geometric standard deviation

Table 2 Statistical analysis (Dunnett's test) comparing ratios of individual target organs vs. combined data.

Liver vs. combined	Kidney vs. combined	Blood vs. combined	Body weight vs. combined
n.s.	n.s.	$P < 0.05$	n.s.

n.s. = not significant

differences between the respective GMs of the ratios for effects involving the liver, the kidney, or body weight, nor for the GM for the combined data. Table 2 shows the results of the Dunnett's test.

DISCUSSION

We have performed an analysis of previously reported toxicology data to determine assessment factors for four different target organs. In the present study, the ratio derived from the combined data corresponds to the results of previous studies. If the central estimation is used as an assessment factor, our findings from the combined data show that the sub-acute toxicity study NO(A)EL (mg/kg/day) should be divided by 2.4 in order to extrapolate to the sub-chronic toxicity study NO(A)EL. Furthermore, under the consideration of variety of substances, the sub-acute toxicity study NO(A)EL should be divided by 11 or 19 to span the 90th or the 95th percentiles (respectively) of the expected distribution of values. We note that a factor of 3 is used in the EU guidelines (e.g., Falk-Filipsson *et al.*, 2007), and is recommended in ECHA (2012) for extrapolation from sub-acute to sub-chronic exposure. For our results, such a factor of 3 would cover 57% of all substances. Our results are consistent with those of Malkiewitcz *et al.* (2009), who reported a GM (GSD) of a distribution of the NOAEL ratios of 2.7 (2.4) for non-lethal endpoints; those researchers concluded that a factor of 3 would cover 60% of all substances.

Regarding our organ-specific data, we did not observe significant differences between the respective GMs of the ratios for effects involving the liver, the kidney, or body weight compared to the GM for the combined data. Thus, no positive reason was found for the three target organs' effects to be treated separately from the analysis result of the combined data. The implication is that, in these cases, the analysis result obtained from the combined data can be used. On the other hand, our results indicate that the effect on blood must be treated separately. However, few data samples were available related to the effect on blood, making it difficult to discern why only the blood effect was distinct. The specific manner in which this effect occurs will need to be addressed in further analyses.

Our results also relate to refinement of assessment factors. Assessment factors related to exposure duration generally take into consideration the following two results of increased exposure duration: (a) similar effects appear at a lower dose, and (b) other effects may appear (e.g., Groeneveld *et al.*, 2004). If the contribution of (b) is dominant, then there exists a target organ such that the absolute value of the slope of the relation between exposure duration and NO(A)EL is greater than the result from the combined data (as seen in Fig. 2). However, in our analysis, no individual target organs yielded a ratio exceeding the result from the combined data. Therefore, the contribution of (b) does not appear to dominate, and it may be surmised that the contribution of (a) could be substantial.

We note that subsequent analyses will need to consider differences in experimental study design between sub-acute and sub-chronic studies, such as contrasts in dose-spacing and the number of test animals. According to OECD Guidelines for Testing of Chemicals No. 407 and No. 408 (OECD, 2008, 1998), in the case of sub-acute studies, dose groups should consist of at least 10 animals (five animals per sex), and dosage selection with a common ratio of two to four is optimal; in the case of sub-chronic studies, these guidelines indicate that dose groups should consist of at least 20 animals (10 animals per sex), again with a dosage selection of two to four. With respect to dosage selection, the means of common ratios of dosage selection were 3.2 and 3.5 with respect to sub-acute and sub-chronic studies, respectively, in the data used in the present study. Therefore, the problem with respect to dose spacing is not expected to have been signifi-

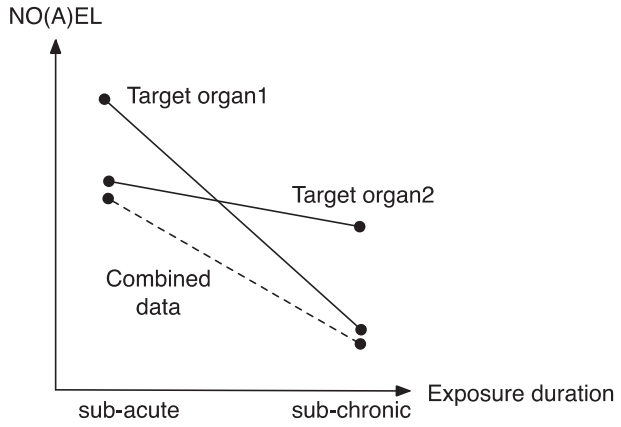

Fig. 2. Hypothetical diagram demonstrating possible relationship between sub-acute NO(A)EL and sub-chronic NO(A)EL for individual target organs (internal organ or body weight). In this example, the absolute value of the slope of the correlation between exposure duration and NO(A)EL for Target organ1 exceeds the value derived from the combined data.

cant in our analysis. Also, OECD guidelines do not distinguish between sub-acute and sub-chronic studies. On the other hand, those guidelines do suggest a difference in the number of animals used in experimental studies for sub-acute and sub-chronic studies. In the data used in the present study, the means of the number of test animals in each dose group used in the experimental studies were 8.3 and 14 for sub-acute and sub-chronic studies, respectively. Additionally, OECD guidelines recommend that sub-chronic studies use twice as many animals in each dose group compared to sub-acute studies. However, the present study did not consider differences in the number of animals used in individual experimental studies. Statistical quantification and examination of the possible impact of differences in the number of test animals should be the subject of future analyses.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Makoto Ema for his support and fruitful discussion in improving this paper. This study was supported in part by JSPS KAKENHI Grant Number 25871184.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Bokkers, B.G.H. and Slob, W. (2005): A comparison of ratio distributions based on the NOAEL and the benchmark approach for subchronic-to-chronic extrapolation. Toxicol. Sci., **85**, 1033-1040.

ECETOC (2003): Derivation of assessment factors for human health risk assessment, Tech. Rep. 86, European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.

ECHA (2012): Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response for human health, European Chemicals Agency, Helsinki.

Falk-Filipsson, A., Hanberg, A., Victorin, K., Warholm, M. and Wallén, M. (2007): Assessment factors — Applications in health risk assessment of chemicals, Environ. Res., **104**, 108-127.

Groeneveld, C.N., Hakkert, B.C., Bos, P.M.J. and de Heer, C. (2004): Extrapolation for exposure duration in oral toxicity: A quantitative analysis of historical toxicity data. Hum. Ecol. Risk Assess., **10**, 709-716.

Kalberlah, F., Föst, U. and Schneider, K. (2002): Time extrapolation and interspecies extrapolation for locally acting substances in case of limited toxicological data. Ann. Occup. Hyg., **46**, 175-185.

Kalberlah, F. and Schneider, K. (1998): Quantification of extrapolation factors, Final report of the research project No. 116 06 113 of the Federal Environmental Agency.

Lehman, A.J. and Fitzhugh, O.G. (1954): 100-Fold margin of safety. Assoc. Food Drug Off. U.S.Q. Bull., **18**, 33-35.

Malkiewicz, K., Hansson, S.O. and Rudén, C. (2009): Assessment factors for extrapolation from short-time to chronic exposure — Are the reach guidelines adequate? Toxicol. Lett., **190**, 16-22.

NITE (2010): The initial risk assessment report. Available at http://www.safe.nite.go.jp/english/risk/initial_risk.html.

OECD (1998): OECD guidelines for the testing of chemicals, Section 4, Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. Organisation for Economic Co-operation and Development, Paris.

OECD (2008): OECD guidelines for the testing of chemicals, Section 4, Health effects, Test No. 407: Repeated dose 28-day oral toxicity study in rodents. Organisation for Economic Co-operation and Development, Paris.

Renwick, A.G. (1991): Safety factors and establishment of acceptable daily intakes. Food Addit. Contam., **8**, 135-149.

Renwick, A.G. (1993): Data-derived safety factors for evaluation of food additives and environmental contaminants. Food Addit. Contam., **10**, 275-305.

Schneider, K., Hassauer, M., Oltmanns, J., Schuhmacher-Wolz, U., Elmshäuser, E. and Mosbach-Schulz, O. (2005): Uncertainty analysis in workplace effect assessment, Research Report F1824, F1825, F1826, Federal Institute for Occupational Safety and Health Dortmund Germany, Dortmund.

US EPA (1988): Recommendations for and documentation of biological values for use in risk assessment, EPA 600/6-87/008, US Environmental Protection Agency, Washington, DC.

US EPA (2002): A review of he reference dose and reference concentration processes, EPA/630/P-02/002, US Environmental Protection, Washington, DC.

WHO/IPCS (1994): Assessing human health risk of chemicals: derivation of guidance values for health-based exposure lim-

its, Environmental Health Criteria, No. 170, International Program on Chemical Safety, WHO/UNEP/ILO, World Health Organization, Geneva.

WHO/IPCS (1999): Principles for assessment of risks to human health from exposure to chemicals, Environmental Health Criteria, No. 210, International Program on Chemical Safety, WHO/UNEP/ILO, World Health Organization, Geneva.

WHO/IPCS (2005): Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in Dose/concentration-response assessment, International Program on Chemical Safety, WHO/UNEP/ILO, World Health Organization, Geneva.

Woutersen, R., Til, H. and Feron, V. (1984): Sub-acute versus subchronic oral toxicity study in rats: Comparative study of 82 compounds. J. Appl. Toxicol., **4**, 277-280.

Yamada, T., Hasegawa, R., Sakuratani, Y., Yamada, J., Yoshinari, K., Yamazoe, Y., Hirose, A. and Hayashi, M. (2013): Hazard evaluation support system (HESS) II. Comprehensive data analysis; distribution of NOEL, organ toxicity and mechanistic information for categorization. J. Toxicol. Sci., **38 Supplement**, P-118.