



A New Way in Deciding NOAEL Based on the Findings from GLP-Toxicity Test

Yeong-Chul Park¹ and Myung-Haing Cho²

¹GLP Center, Catholic University of Daegu, Kumrak 5-Ro, Hayang-Up, Kyongsan-Si, Kyongbuk 712-702, Korea

²Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

(Received July 2, 2011; Revised July 16, 2011; Accepted August 11, 2011)

The FDA guidance focuses on the use of the NOAEL to establish the maximum recommended starting dose. The majority of NOAEL has been described inaccurately or incompletely in final reports for 90-days repeated dose toxicity test based on GLP (good laboratory practice) regulation. This is the most serious one of reasons for why most pharmaceutical companies targeting global markets have disregarded the final report produced from GLP facilities in Korea. The problems in deciding NOAEL reflected in the final reports are mainly due to the followings; 1) Inaccurate description or use of NOEL, NOAEL and LOAEL, 2) Insufficient and inappropriate interpretations in findings from toxicity test. This paper is intended to provide the insight into distinguishing NOAEL from NOEL and LOAEL, and into classifying findings from toxicity test. Here, the three step method is newly suggested by applying the weight-based classification to the NOEL, NOAEL and LOAEL based on the findings.

Key words: GLP, NOAEL, Adverse effects, Weight-based classification, Non-carcinogenic test

In drug development, the greatest obligation is protecting the safety of volunteers and patients in clinical trials. For this purpose, drug companies tend to perform a fairly standard package of nonclinical studies before commencing First-In-Human (FIH) clinical trial investigations. The US FDA 'Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers' addresses the use of the maximum recommended starting dose (MRSD) for the FIH clinical trial (US FDA, 2006). In addition, the FDA guidance focuses on the use of the NOAEL (no observed adverse effect levels) to establish MRSD.

In general, NOAEL is determined in various toxicity tests performed in the most sensitive and relevant animal species. Many toxicity tests examine specific types of adverse effects, known as "endpoints," such as clinical and pathological findings. Other tests are more general in nature, ranging from single-exposure ("acute") studies to multiple-exposure ("repeat dose") studies, in which animals are administered daily doses of a test substance to calculate NOAEL and determine whether one or more organ or system is adversely

affected following exposures of one-month ("subacute"), three-month ("subchronic"), and/or two-year ("chronic") duration.

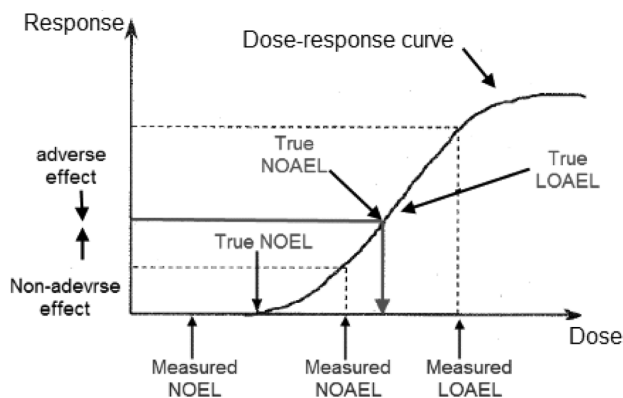
In Korea, exposure test of three-month, called 90-days repeated dose toxicity test, has been popularly used to calculate NOAEL. However, the majority of NOAEL has been described inaccurately or incompletely in final reports for 90-days repeated dose toxicity test based on GLP (good laboratory practice) regulation. This is the most serious one of reasons for why most pharmaceutical companies targeting global markets have disregarded the final report produced from GLP facilities in Korea. The problems in deciding NOAEL reflected in the final reports are mainly due to the followings; 1) Inaccurate description or use of NOEL (No observed effect level), NOAEL and LOAEL (lowest observed adverse effect level), 2) Insufficient and inappropriate interpretations in findings from toxicity test. This paper is intended to provide the insight into distinguishing NOAEL from NOLE and LOAEL, and into classifying findings from toxicity test.

Traditionally, the view has been that non-cancer and non-genotoxic cancer endpoints are assumed to express NOEL, NOAEL and LOAEL as defined in Table 1. In most final reports for 90-days repeated dose toxicity test produced from GLP facilities in Korea, the NOEL and NOAEL has been expressed identically as 무해용량 or 무영향용량 in Korean.

Correspondence to: Yeong-Chul Park, GLP Center, Catholic University of Daegu, Kumrak 5-Ro, Hayang-Up, Kyongsan-Si, Kyongbuk 712-702, Korea
E-mail: ycpark@cu.ac.kr

Table 1. Definitions and Korean expression for NOEL, NOAEL and LOAEL

Evaluation endpoints	Definitions	In Korean
NOEL	The highest exposure level at which there are no effects (adverse or non-adverse) observed in the exposed population, when compared with its appropriate control.	최대무영향용량
NOAEL	The highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. Some effects may be produced at this level, but they are not considered to be adverse or precursors to adverse effects.	최대무독성용량
LOAEL	The lowest exposure level at which there are statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control.	최소독성용량

**Fig. 1.** Quantitative outcome of toxicity studies dependent upon observation points.

As shown graphically in Fig. 1, it is apparent to resolve the confusion, a clear form of words that describes the terms NOEL, NOAEL and LOAEL (Lewis *et al.*, 2002). They are needed along with separate definitions of the phrases “adverse effect” and “biologically significant”. The NOAEL, 최대무독성용량, is not the same as NOEL, 최대무영향용량, which refers to any effect, not just an adverse effect. The definition of the NOAEL, in contrast to that of the NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern. In addition, the NOAEL should also not be confused with LOAEL, 최소독성용량, which refers to adverse effects. Agreement on these definitions is fundamental to development of coherent criteria that can be used to differentiate adverse from non-adverse effects. Thus, it is important to note that GLP facilities in Korea will actually be employing NOAEL in their final report for 90-days repeated dose toxicity test. Also, this employment is satisfied with the FDA guidance recommending the use of the NOAEL to establish MRSD.

The second problem in deciding NOAEL reflected in the final reports produced from GLP facilities in Korea is due to insufficient and inappropriate interpretations in findings from toxicity test. These data interpretations play a critical role in determining ultimate risk for test substance. How-

ever, it seems that the test design, especially including dose ranging for 90-days repeated dose toxicity test has been focused on producing no toxicity in the test. This tendency would come from that most study directors want to decide the NOEL easily in the final reports produced from GLP facilities in Korea. In addition, a lot of findings including non-adverse or adverse effects in study results has been negligible in the final reports. This tendency seems to be due to some difficulties in distinguishing between adverse and non-adverse effects in findings from the test. Thus, it needs to categorize each individual finding within a study. Especially, this approach will be helpful for deciding the NOAEL instead of the NOEL.

It was suggested that three categories of “important”, “minor compound-related”, and “noncompound-related” to assign the appropriate weight to individual findings, called “weight-based classification” (Ness, 2006). The weight-based classification is the integrated analysis across multiple endpoints that is relied upon to assess whether a given exposure is adverse. The determination of “adverse” relies on the judgment made by considering the impact of all treatment effects in the study on the health of the individual animal. Thus, how to decide the criteria for non-adverse and adverse effects according to practical findings from the test will be the first step.

The terms “adverse” and “toxicity” are often used interchangeably. An adverse effect may be defined as a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism’s ability to respond to an additional environmental change. On the other hand, an adverse effect may be considered as any change from an organism’s normal state that is irreversible at least irreversible during exposure or following cessation of exposure. Contrasted to adverse effects, non-adverse effects is defined the absence of changes in morphology, growth, development and life span (US National Academy of Sciences, 1975). As shown in Fig. 1, however, non-adverse effect ranged in the initial part of dose-response curve. This means that there are some effects caused by exposure. These effects are reversible following cessation of exposure without detectable impairment

of the organism to maintain homeostasis, and do not enhance susceptibility to the deleterious effects of other environmental influences. Thus, non-adverse effect will be mild and reversible. This is a good criteria to support that the NOAEL is not the same as NOEL. However, it will often not be readily possible to differentiate between adverse and non-adverse effects from routine toxicity tests. Thus, it needs to set up the criteria for real findings acquired from toxicity test in order to differentiate between adverse and non-adverse effects. The following findings in routine toxicity tests can be the criteria as adverse effects; 1) findings showing an obvious dose response at all treated dosages or higher dosages in clinically pathologic observations or histopathologic observations not shown in normal control, 2) findings showing histopathologic lesions which is not shown in normal control and is coincident with statistical and biological significance in clinically pathologic observations at any treated dosages. In a case of non-adverse effects, the following findings in routine toxicity tests can be the criteria; 1) findings showing an weak dose response at all treated dosages in clinically pathologic observations or histopathologic observations shown in normal control. Thus, the findings to set up the criteria for adverse and non-adverse effects depend on whether the clinically pathologic and histopathologic endpoints are shown in normal control or not. In addition, the strength of dose-response and the pharmacological effects can be considered.

In the second step, the findings from clinically pathologic observations or histopathologic observation can be classified based on the weight-based classification. An important compound-related change, signifies that it: 1) is adverse, 2) is part of a constellation of changes that is adverse, 3) reflects a known target organ toxicity for the compound, even if the magnitude in the present study is not profound. "Minor compound-related changes" are effects due to the compound but do not contribute to the characterization of the toxicity profile. These may be of such low magnitude as to be considered biologically irrelevant or may reflect desirable pharmacological properties of the compound. Lastly, "noncompound-related changes" generally fall outside the control range in the study, may be adverse or non-adverse, but are not considered compound-related due to lack of dose response or consistency with historical data.

Now in the final step, the weight-based classification can be used to decide NOEL, NOAEL and LOAEL as follows;

- If there is an important compound-related change in findings, the lowest dose is evaluated as the LOWEL.
- If there is a minor compound-related change in find-

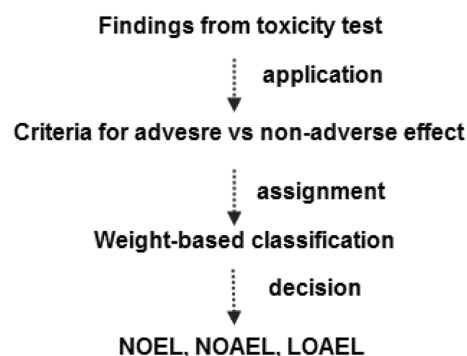


Fig. 2. Proposed scheme for deciding NOEL, NOAEL and LOAEL.

ings, the highest dose is evaluated as the NOAEL.

- If there is a noncompound-related changes without any important compound-related change or minor compound-related change, the highest dose is as the NOEL.

These has been some problems to decide the NOEL, NOAEL and LOAEL based on the findings in final reports for 90-days repeated dose toxicity test produced from GLP facilities in Korea. These problems were mainly due to the inaccurate understanding for the NOEL, NOAEL and LOAEL, and due to inappropriate data-interpretation. Here, as shown in Fig. 2, the three step method is newly suggested by applying the weight-based classification to the NOEL, NOAEL and LOAEL based on the findings. These approach would be helpful to overcome some problems in final reports. However, the further considerations must be necessary in a view of how to classify the findings in more detail to differentiate between adverse and non-adverse effects.

REFERENCES

- Lewis, R.W., Billington, R., Debryune, E., Gamer, A., Lang, B. and Carpanini, F. (2002). Recognition of adverse and nonadverse effects in toxicity studies. *Toxicol. Pathol.*, **30**, 66-74.
- Ness, D. (2006). Writing the nonclinical study final report: A focus on compliance, accuracy, and scientific soundness. *2006 issue of APO*, **7**, 36-41.
- US FDA. (2005). Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Pharmacology and Toxicology.
- US National Academy of Sciences. (1975). Principles for Evaluating Chemicals in the Environment-. A Report of the Committee for the Working Conference on Principles of Protocols for Evaluating Chemicals in the Environment. National Academy of Sciences, National Research Council, Washington, DC.