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Compilation and use of genetic toxicity historical control data

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ABSTRACT

The optimal use of historical control data for the interpretation of genotoxicity results was discussed at the 2009 International Workshop on Genotoxicity Testing (IWGT) in Basel, Switzerland. The historical control working group focused mainly on negative control data although positive control data were also considered to be important. Historical control data are typically used for comparison with the concurrent control data as part of the assay acceptance criteria. Historical control data are also important for providing evidence of the technical competence and familiarization of the assay at any given laboratory. Moreover, historical control data are increasingly being used to aid in the interpretation of genetic toxicity assay results.

The objective of the working group was to provide generic advice for historical control data that could be applied to all assays rather than to give assay-specific recommendations. In brief, the recommendations include:

1. The experimental protocol should remain fixed throughout the period during which the historical control data relevant to the current experiment are being built up, unless it can be demonstrated that changes to the protocol have not affected the values.
2. All data (both individual and group mean values) should be accumulated.
3. No negative control values (i.e., vehicle/solvent controls and absolute/culture medium controls, when available) should be eliminated from the data set, even if considered unusual, unless there is a scientifically justified reason, such as when they were obtained by an identified technical error. However, experiments may need to be repeated if disqualified by historical control data.
4. A minimum set of data resulting from at least 10, preferably 20, independent experiments is recommended to create the historical data set, depending upon the complexity of the assay.
5. It is not appropriate to use the simple range (minimum and maximum value observed during the data accumulation period) of the accumulated historical, especially negative, control data for an assessment. Rather, the distribution of the data together with appropriate descriptive statistics should be considered (e.g., confidence intervals, 95–99% percentiles).
6. For an experiment, when statistically significant increases over the concurrent negative controls (i.e., vehicle/solvent controls) are comparable, i.e., within confidence intervals, with the negative historical data, the biological importance needs to be carefully considered.
7. Historical control data could potentially have an important role in the future to help interpret aspects of genotoxicity data, such as dose response relationships.

1. Introduction

For genotoxicity testing, historical control data represent an important instrument for quality control as well as for data interpretation. The use of historical control data in the interpretation

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of genotoxicity results was identified as an important topic during the 2005 International Workshop on Genotoxicity Testing (IWGT) [1], but was not discussed in depth at that time. The topic was carried over to the 2009 IWGT meeting, with the objective of discussing the optimal use of historical control data in more detail. The historical control working group mainly discussed negative control data although positive control data were also considered to be important. Historical control data are at present mainly used in comparisons with the concurrent control data as part of assay acceptance criteria. Historical control data, however, are increasingly used in the interpretation of results.

Despite their importance in routine testing, clear guidance on how to use historical control data has not been available, though some have provided recommendations. Hayashi et al. [2] used a large set of *in vivo* micronucleus test data generated in the same laboratory to illustrate the importance of historical control data. They analyzed data distributions and used quality control charts to explore changes over time in the pattern of negative control data. Helpful recommendations were published on historical control data to be used in *in vivo* genotoxicity tests [3]. These two publications provided the basis of the IWGT 2009 discussions, with the working group considering whether the recommendations in the papers were still acceptable, and how they could be widened to include the full range of genotoxicity assays including *in vitro* tests.

The aim of the group was not to produce assay-specific recommendations, but rather to provide generic advice for historical control data that could be applied basically to all assays, and to achieve consensus on answers to the following questions:

1) What are historical control data?
2) How is an historical control data base created?
3) How are historical control data used?

2. What are historical control data?

The working group mainly focused on negative historical control data (i.e., vehicle/solvent controls and absolute controls, when available). Historical control data can be defined as the complete set of control data from previous experiments, excluding only those where a technical error was identified in obtaining them. All experiments must have concurrent negative control(s) and all concurrent negative controls should be combined to produce the historical negative control data. The experimental unit for statistical analysis is usually the animal for *in vivo* assays, and the culture/plate for *in vitro* assays, even if the incidence of genetic damage is generally expressed per cell.

Historical negative controls should be compiled separately for species, strains, tissues, cell types, treatment and sampling times as well as vehicles within each laboratory. Measurements should be defined for each assay by the scientific community. These are usually measurements which describe the spontaneous or background rate of genotoxicity (e.g., mutant frequency, incidence of micronucleated cells, incidence of chromosome damage per cell), and, in some cases, cell proliferation parameters (e.g., survival growth, mitotic index, ratio of polychromatic to normochromatic erythrocytes).

3. How is an historical control data base created?

All historical data should initially be accumulated to create the historical control data base. The distribution of the data is an important feature of the historical control data. Reporting only the range, i.e., the highest and lowest observed values, is not considered adequate. Data should therefore not be excluded simply because it is unusual and shows a large deviation from the accumulated data, as this could be a reflection of the assay variability. Data should only be rejected when there is a clear, valid and scientifically justified reason to do so.

It is important that the experimental protocol remained unchanged through the period covered by the collected historical data, including the current experiment, unless it can be shown that any changes have not affected the values. The following factors should be kept unchanged during the accumulation of the control data for the historical control set [4]:

1. The experimental protocol should remain constant throughout the period covered by the historical data and the current experiment. If changes occur in the experimental conditions, e.g., chemical and equipment suppliers, treatment and sampling time, it is important to check the absence of impact on the historical data. Differences between the new negative control data with the ones accumulated before the change in the experimental conditions would be evaluated with the method in place to compare treated and negative control samples.
2. The method of scoring should be unchanged during the period.
3. The experimental units (e.g., cells and animals) should remain comparable throughout the period.
4. No known systematic differences should exist between the various control groups, current and historical, which might produce systematic differences in response, e.g., data should be collected in a consistent manner within the same laboratory.

Provided that the criteria necessary for ensuring that the quality of the historical control data are met, then these data can be combined, e.g., over vehicles and time. There should be no systematic differences between the various current and historical control groups [5].

The historical control data set should be updated regularly and, ideally, should be as large as possible, and from at least 10 separate experiments, but preferably 20. The minimal number, however, may depend on the use to be made of the historical data (e.g., acceptance criteria, analysis of distribution, internal quality control), the variability within an assay, and also practicability (e.g., number of assays per year). The data set should be analyzed in a way that ensures that the more recent data carries greater weight than older data. Analysis of data distribution and internal quality control generally require a large number of data and should be conducted with all accumulated data, while acceptance criteria could be based on the 10–20 most recent experiments.

4. How are historical control data used?

4.1. Assay design

The historical negative control data are important for the design of an assay. Assays should be designed (e.g., number of duplicates, number of cells scored) to detect a biologically important increase over the background, which should be predetermined by the researchers carrying out the study and be consistent with accepted criteria defined for this assay. The experimental design should be based on statistical considerations [6]. It should be based on the α-level for recognizing a predetermined increment over the spontaneous level with an acceptable statistical power (1minusType II error). A design that is able to detect, at an α-level (type I error) of 0.05, a predetermined difference in the endpoint from the background level (which is a test specific value) with a power of 80% was considered to be an achievable minimum standard. In some assays, a doubling of the background level is used, however, such a fold-based approach may have different biological meaning for different assays as well as depending on the back-

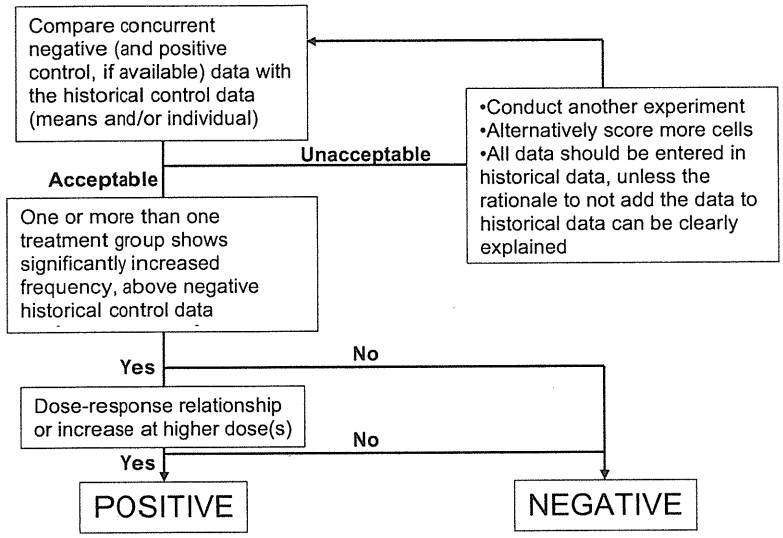

Fig. 1. An example of a strategy to evaluate assay results including historical control data.

ground level. Moreover, the appropriate statistical analysis should be based upon the experimental design. Statistical significance and biological importance should be compatible rather than contradict each other.

A clear description of the design of the experiment, including the power of the test, the statistical methods used to analyze the data, and the level of significance actually achieved should be given in every study report.

The sample size should be large enough to ensure that at least one adverse event (e.g., micronucleus, aberrant cell, dead implant), if induced, will be recorded for each experimental unit. In the *in vivo* assays, the experimental unit for statistical comparisons is the animal. Limited animal resources and ethical considerations mean that it is important to design the experiment with the optimal number of animals based upon statistical considerations. Similarly, for *in vitro* assays, the study should be optimized (e.g., number of plates, cultures, cells scored) in order to achieve adequate test sensitivity and the optimum use of resources.

4.2. Data distribution, parameters and statistical analysis

Data should be collected to determine the distribution of the data for individual and the group means, and to calculate the important descriptive statistics to be used in data analyses. The assay-specific choice of the appropriate statistics, such as mean or median for the endpoints, to be used in the assessment of historical control data should take into consideration what has been agreed upon by the scientific community in case of clear consensus, and published recommendations based upon their experience. Graphical control charts can be helpful for exploring datasets to identify trends such as can arise from assays 'drifting'.

The use of the range of the historical data (i.e., minimum and maximum value observed during the data accumulation period) is not considered appropriate, but rather the confidence interval (e.g., the 95th percentile) is preferred for describing historical data.

Outlier values are problematic and more work may be needed to aid in their identification for specific assays. Historical control data can be used by expert groups for each assay to define acceptable "ranges", e.g., as has already been done for the mouse lymphoma TK assay (MLA; [3]). Experimental data should not deviate greatly from data reported in the literature. If decisions were to be based solely upon "ranges" then outliers might have disproportionate effects. This is one reason why the use of ranges is not recommended, and approaches using confidence intervals are preferred. In the event of an outlier, the animal(s) or culture(s) responsible for the differences from the negative control data should be identified. It could then be eliminated in case of good scientific rationale. If no rationale is identified, it will be added to the historical data base, and will be taken into account for the analysis of data distribution.

4.3. Quality control and test acceptance criteria

The main approaches to using negative historical control data in the assessment of study results are to check whether the concurrent control data are consistent with the historical negative control data collected in the same laboratory. Each assay will have its own specific acceptance criteria. These criteria, based upon a series of negative controls results, are also important in assessing whether the assay is still under "control" within a laboratory. This can be done using formal quality control methods. Historical control data are also important in ensuring that the laboratory has established familiarization and technical competence with the assay.

If a negative control group in an experiment falls outside the acceptance criteria, then the experiment is no longer valid. Some use may be made of the information from the experiment if, for instance, the treated group shows a clear increase. However, care is needed because such an increase may be the result of an artifact. For instance, a problem with the culture conditions in an *in vitro* study could have affected all the cultures and the whole experiment. In the case of a negative result, the experiment would have to be repeated. Even if data fall outside the acceptable range, it should be included in the historical data set unless exclusion can be scientifically justified, such as, e.g., being the consequence of a technical artifact.

Each assay needs to define its own acceptance limit/criteria in order to be able, based upon the historical control, to reject a control group and, therefore, the experiment. These could be based upon either the observed distribution of the data or calculated from the distribution values using, for instance, the criteria of being outside the range of 2 or 3 standard deviations from the mean of what was actually observed, or outside an upper 99% percentile. The choice of criteria should be determined for each individual assay based upon the specific characteristics of the assay. The acceptance/limit criteria for each assay should be consistent with historical data

generated by the scientific community. It could, for example, be useful to define performance standards and an acceptable range or confidence intervals for each assay as has been done for the MLA (i.e., global evaluation factor [4]), or a level of 5% for chromosomal aberrations [7].

The historical positive control group is also important although it depends on its objective. Often its purpose is to show that a laboratory can identify the effect of a known positive compound. The objective would be to show that the technical factors associated with each specific assay have been done satisfactorily. Each assay should, therefore, define acceptance criteria for positive control data; however, formal statistical methods for assessing a set of historical positive control data may not be needed.

4.4. Interpretation of results

The OECD test guidelines on genotoxicity tests stress biological importance in evaluating the test results. This does not mean that consideration of the statistical analysis of the experiment is not needed for the evaluation. In fact, the statistics used for each assay type should be chosen in a way that ensures the biologically important effects can be adequately detected. The historical control data should play an important role in the statistical evaluation of the experimental data as well as helping to understand the nature of specific tests data. Fig. 1 shows an example of the assessment of data including the use of historical control data, together with the analysis of the dose–response relationship using the concurrent negative control data.

The working group noted that both concurrent negative controls and historical control data are essential in the assessment of the biological importance of experimental data. The assessment should be considered as a two-step process: first, a comparison of the experimental data with the concurrent negative control, then second, a consideration of whether the results are meaningful when compared with the distribution of the historical data. For example, when statistically significant increases over the concurrent negative control data are detected, but the values remain within the historical control data range (i.e., within the confidence interval for the historical control data), then the biological importance of the statistical increase needs to be carefully considered to judge whether the increase is biologically meaningful. It should be noted that if means and individual values are both accumulated as the historical control, the comparison should be made between the observed group means and the historical control group means, and the individual values for both data sets.

Historical control data can have an important role in defining the size of effect that is considered biologically important. Such considerations may have a role in the future in putting the dose-response relationship seen in experimental studies into perspective in the context of what a meaningful biological effect level may be. Considerable care, though, would be needed in the choice of data to use in such assessments and more work will be needed to assess the potential of this approach.

5. Conclusions

Historical control data are an integral part of routine assay conduct and interpretation in genotoxicity testing. It is important that the experimental protocol remains constant during the period of the construction of the historical control data set, and that the current experiment uses the same protocol, unless it can be demonstrated that the change does not affect the values. Historical control data are important for developing the acceptance criteria used to evaluate the technical acceptance of the assay and also, using appropriate statistical analysis of the data, for the evaluation of the test result. For these purposes, the simple range (minimum and maximum values observed during the data accumulation period) of the accumulated data is not adequate; instead the distribution of the data should be used in the assessment.

Conflict of interest

None declared.

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