Historical Control Distribution (HCD) Working Group Survey

The GTTC HCD Working Group is querying laboratories about their experiences with compiling, maintaining and using historical control data and distributions for genetic toxicology assay acceptance and evaluation. The compiled data will be used to determine where consensus approaches exist and, where possible, make recommendations on compiling, maintaining and monitoring historical control data and distributions.

Whilst we would like your experience for all genetic toxicology assays, if this is not possible in the timeframe given, we ask that you provide information for the most commonly used assays as noted in the Excel-based survey. Although we would be interested to know if different approaches are used for the most commonly used/routine assays versus those that are not performed routinely in your laboratory, if the same approach for compiling, maintaining and monitoring historical control data and distributions (i.e., the same answers apply) for multiple assays / endpoints, please indicate which assays are applicable in the survey.

Please use this document as a reference as you fill-out the Excel-based survey (available for download **here**). Ensure that the **macros are enabled** (otherwise you may not be able to select multiple responses for the relevant questions). Completed surveys should be sent to Connie Chen (cchen@hesiglobal.org) by **April 1, 2022**.

If you have any questions regarding the survey, please reach out to the project co-leads Robert Smith (robert.p.smith@labcorp.com) and Robert Heflich (robert.heflich@fda.hhs.gov).

TAB 1: GENERAL QUESTIONS

Contact Details *(Note: This information will be blinded as results are collected)*

- Name:
- Laboratory:
- Email:

May the GTTC HCD WG contact you with additional and/or clarifying questions?

- Yes
- No

Do you consider use of Historical control data and distributions as critical for assay evaluation of genetic toxicology study data independent of test guideline requirement?

- Yes
- No
- *Comments (free text)*

What do you consider the usefulness of historical control distributions to be? *(Several answers can be chosen)*

- A reference interval / range to flag outliers during study data evaluation
- A measure of the biological noise for the assay
- A measure of the normal background variability for the assay
- Used for comparison to study controls to assess study reliability
- Used for comparison to study controls to identify drift in data / species / strain
- A replacement for concurrent control data
- Used alongside statistical analysis of study results (such as pairwise comparison or trend tests) for overall evaluation of study data
- Used for assessment of the biological relevance of statistically significant increases in study data
- An indication of how well the laboratory is performing the assay
- Never consider HCDs
- Other purpose than listed, please specify in 'Comments'
- *Comments (free text)*

Do you compare your laboratory HCDs against other laboratory's HCDs or published vehicle control data, for example?

- Yes
- No
- *Comments (free text)*

If no, would you see benefit from a 'global' HCD for the assay / endpoint, similar to the use of a predefined induced mutant frequency (known as the Global Evaluation Factor (GEF)) to define positive and negative responses for the Mouse Lymphoma Assay (MLA)?

- Yes
- No
- Comments: *(free text)*

In your opinion, what can limit the use of historical control data when available? *(Several answers can be chosen)*

- Insufficient information on the criteria for selecting historical control data (e.g. number of studies, number of replicates, study conditions, etc.)
- Insufficient level of details in reporting historical control data
- Insufficient number of studies to source historical control data
- Insufficient number of replicate values included in the historical control data set
- Lack of consistent terminology
- Lack of consistent format
- Lack of guidance on how to use, report and interpret historical control data
- Lack of information related to the methods and settings used to collect the data
- Lack of information on how HCDs are generated
- Nothing
- Other, please specify in 'Comments' below.
- Comments: *(Free text)*

What best describes the way your laboratory handles statistical analyses, including HCD:

- Study directors are responsible for statistical analyses, and their resources are primarily SOPs and scientific literature
- We outsource for statistical guidance and specific analyses as necessary
- In-house statistician(s) provide guidance and oversee analyses related to genotoxicity studies

What type of data do you use to derive your HCD?

- GLP
- Non-GLP
- Both
- Comments

Do you think non-GLP data can be included in establishing historical control distributions?

- Yes
- No
- Comments

If the GTTC HCD WG were to perform a collection of historical control data, would you be willing to share historical control data?

- Yes
- No

TAB 2: ASSAY SPECIFIC QUESTIONS

Columns are provided for the following assays/endpoints. Please fill in each column as appropriate. You may delete columns for the assays/endpoints you will not be

Assay / Endpoint Assessed:

- **In vivo:**
    - Bone Marrow / Peripheral Blood Micronucleus
    - Comet Assay
    - Pig-a Assay
    - Transgenic Rodent Gene Mutation Assay
- **In vitro:**
    - Ames
    - In vitro Micronucleus
    - tk Gene Mutation Assay
    - HPRT Gene Mutation Assay
    - In vitro Chromosome Aberration

Scoring method *(Select all that apply)*

- Manual scoring
- Image analysis
- Flow cytometry
- Comments: *(Allow free text)*

Experience of performing the assay?

- <1 year
- 1 – 2 years
- 2 to 5 years
- 5 to 10 years
- >10 years

Approximate number of experiments per year?

- <10
- 11 to 20
- 21 to 40
- >40

Historical Vehicle Control Database *(A historical control database is compiled and used to determine the historical control distribution)*

Are group mean values or individual replicate values included in the historical control database to determine vehicle control HCDs? *(Note: individual replicate refers to the experimental unit, which is usually the animal for in vivo assays, and the culture/plate for in vitro assays)*

- Individual replicate values (*please state number of replicates included per experiment in 'Comments' below*)
- Mean values
- Both (i.e. separate HCDs determined for both individual and group means)
- Comments: *(Allow free text)*

Why are mean / individual values used in your laboratory to determine HCDs?

- Historically done this way
- Acceptance / evaluation criteria
- Benefits discussion of results
- Other: *(Allow free text)*

What is the basis for how many data points are included in the historical control?

- Based on number of data points? E.g., 40, 100, 200, etc.
- Based on time? E.g., previous year, 2 years, etc.
- Comments: *(Allow free text)*

What criteria are used to exclude study vehicle control data from the historical control database?
(Several answers can be selected – if criteria are not detailed in the below options, please state your criteria in 'Comments' below)

- Data does not fulfill the study acceptance criteria
- Data does not fall within the current laboratory observed historical control range (i.e. min/max values)
- Data does not fall within the current laboratory calculated laboratory historical control distribution
- Data falls outside 'k' standard deviations (SDs) of current laboratory HCD mean *(state 'k' used in 'Comments' below and how SDs are generated, e.g. from Excel, control charts, etc.)*
- Data is an unusual/extreme value *(state criteria for unusual/extreme' in 'Comments' below)*
- There is a clear, valid and scientifically justified reason to do so *(e.g. technical or obvious mistake. State criteria in 'Comments' below)*
- Statistical methods are used to exclude data from HCD *(State methods used in 'Comments' below)*
- All data is included
- All data included if no technical reason to exclude
- Comments: *(Allow free text)*

If any vehicle control data is excluded from the historical control database (using any of the criteria stated above), do you monitor or investigate quality control?

- Yes (see question below)
- No
- Comments: *(Allow free text)*

How do you demonstrate that the assay is under control? *Examples could include use of acceptance criteria for each experimental occasion, defined criteria (similar to those details above), run charts, control charts, statistical tools, etc.*

- *(Allow free text)*

HISTORICAL VEHICLE CONTROL DISTRIBUTIONS (HCDS)

What range do you use for the historical control distribution?

- The observed range (i.e., the min/max)
- A calculated distribution (e.g., standard deviation(s), point estimates such as parametric limits [confidence/tolerance/prediction interval, etc.], or non-parametric limits such as empirical percentiles)) (*Note: please state what method is used and rationale for why you use this method in the 'Comments' below*
- Comments: *(Allow free text)*

Do you characterize whether the distribution of your historical vehicle control data is normal?

- Yes
- No
- Comments: *(Allow free text)*

Do you perform any statistical transformations on your vehicle control data prior to calculating HCDs?

- Yes
- No
- Comments: *(Allow free text)*

To what extent are HCDs split for the assay / endpoint? *(Select all that apply. Please add additional information in the 'Comments', for example if split per vehicle, is this organic v's aqueous, or per vehicle)*

In Vivo

- Per species
- Per strain
- Per sex
- Per tissue
- Per vehicle
- All data for the endpoint is included in one HCD
- Comments: *(Allow free text)*

In Vitro

- Cell type
- Per strain *(Ames)*
- Plate incorporation v's pre-incubation *(Ames)*
- Per treatment regimen
- w/ and w/o S-9

- Source of S-9 *(i.e. Aroclor v's Phenobarbital/5,6-Benzoflavone induced, species, etc. Please state in Comments; below)*
- Per vehicle
- All data for the endpoint is included in one HCD
- Comments: *(Allow free text)*

What information is presented in HCD tables in a study report. *(Select all that apply. Example table(s) can be provided)*

- Number of experiments
- Number of individual replicates
- Mean
- Median
- 95% Confidence Interval
- Standard Deviation *(Please state in 'Comments' how SD is generated, e.g. excel, control chart, etc.)*
- Observed Range (min/max)
- Calculated distribution (as stated above)
- Date of HCD generation
- Date range of study data included in the HCD
- Information on the stratification or lack of stratification over vehicles, sex etc. (as detailed in above question)
- Comments: *(Allow free text)*

How often are HCDs updated?

- Yearly
- Bi-annual
- After 'x' number of experimental occasions *(Please include number of experimental occasions to 'Comments' section below)*
- After 'x' number of data points *(Please include number of data to 'Comments' section below)*
- Changes to the experimental protocol *(Please comment below)*
- Dependent on the frequency of experimental occasions *(Please comment below)*
- Continuously / After each experiment
- Comments: *(Allow free text)*

Do you determine HCDs for both vehicle control and positive controls?

- Vehicle control HCDs only
- Both vehicle and positive HCDs are generated

Do vehicle control HCDs and positive control HCD overlap? *(If these data overlap, please add further details in 'Comments' on whether this is expected or triggers any action.)*

- Yes, observed ranges overlap
- Yes, observed range and calculated distributions overlap
- No

- Not applicable
- Comments: *(Allow free text)*

USE OF HCDS IN STUDY ACCEPTANCE CRITERIA

Are HCDs used as part of the study acceptance criteria:

- Yes
- No *(if no add further details in 'Comments')*
- Comments: *(Allow free text)*

What historical vehicle control ranges are used as part of acceptance criteria?

- The observed range (i.e. the highest and lowest observed values)
- A calculated range *(If different from method described above please state what method is used in the 'Comments' below)*
- Comments: *(Allow free text)*

How are vehicle HCDs used as part of acceptance criteria of study data? *(Several answers can be selected)* *(Note: Vehicle HCDs are generated from group mean values or individual replicate values have been indicated above, however, this can be further clarified in the comments section below, i.e. whether comparing mean to mean or mean to individual, for example)*

- All individual replicate vehicle control values must fall within the upper and lower values of / fall within the upper value of / be consistent with the observed range (min/max)
- All individual replicate vehicle control values must fall within the upper and lower values of / fall within the upper value of / be consistent with the calculated distribution
- The mean vehicle control value must fall within the upper and lower values of / fall within the upper value of / be consistent with the observed range (min/max)?
- The mean vehicle control value must fall within the upper and lower values of / fall within the upper value of / be consistent with the calculated distribution
- Concurrent study positive control values must exceed the upper value (range or calculated distribution) of the HCD
- Other *(State in 'Comments' section)*
- Comments: *(Allow free text)*

Are positive control ranges used as part of the study data acceptance criteria?

- Yes
- No

Which positive control ranges are used for acceptance criteria?

- Observed ranges (min-max values)
- Calculated distribution
- Statistical significance over concurrent control used, no comparison to historical controls

How are positive control HCDs used as part of study acceptance criteria?

- Not used as part of study data acceptance criteria
- Positive control values must fall within the positive control HCD
- Positive control values must be close /comparable to the positive control HCD
- Comments: *(Allow free text)*

EVALUATION CRITERIA

Are HCDs used as part of the study evaluation criteria:

- Yes
- No

Which vehicle control HCD is used as part of study evaluation criteria?

- The observed range (i.e., the highest and lowest observed values)
- A calculated range *(If different from method described above please state what method is used in the 'Comments' below)*
- Comments: *(Allow free text)*

How are vehicle control HCDs used as part of study evaluation criteria?

- All individual replicate values for a test article treated concentration are compared individually against the vehicle control HCD
- The mean value of replicates for the test article concentration is compared against the vehicle control HCD
- 1 / 2 / 3 / 4 / etc. individual replicate values for a test article treated concentration are compared against the vehicle control HCD
- Other *(State in 'Comments' section)*
  - Comments: *(Allow free text)*

How is the positive control HCD applied for evaluation criteria?

- Not applied?
- Other *(State in 'Comments' section)*
- Comments: *(Allow free text)*