Practical Calculations for QA

OR THE MATH AND SCIENCE YOU ALREADY KNOW

BUT DO WE HAVE TO …?

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GLP Studies – Requirements for Data

Protocols/Amendments

- Define purpose and objectives for data to be collected for the Study

Standard Operating Procedures

- Basis for reliability and performance of equipment and procedures used to generate, collect, and analyze data

Test Methods

- Specifics for analyses of test systems specimens (samples) in a defined, controlled, repeatable manner including acceptance criteria for analyses.

Method Validation

- Testing an analytical method for reliability, ruggedness, specificity/limitations, and other key components to assure analysis method provides data with known levels of confidence and certainty

QA Role in data review/audit and report reliability to study data

- Assigned in US GLPS section .35(b)(6) and OECD PGLP section 2.2.1.d

Key Concepts

Quantitative Analysis - Data expressed as numeric value with units of measure

Dimensional Analysis - Units of measure balanced in calculations

Mass Balance – all data accounted for in calculations

Balanced Equations   left side = right side

Significant Figures – examples:

1000	1	1.000×10^3	4
129	3	5.3×10^2	2
87	2	5.30×10^2	3
0.00536	3	1.8730	5
0.00004	1	0.00004000	4

Significant Figure Rules

Exact numbers are wild card = unlimited sig figs.
- Examples: 60 (minutes/hr) or count like exactly 35 students in a class

All non-zero integers are significant

Zeros before non-zero integers are not significant

Zeros between non-zero integers are significant

Trailing zeros to right of decimal place are significant

Adding/subtracting - least decimal places
- 3.0 + 2.65 + 1.007 = 6.6547 rounded to (RT) 6.7

Multiplying/dividing – least sig figs
- 1000.1/243 = 4.11563786 RT 4.12

Conversions – same # sig figs as started with
- 52.4 in X 1 ft/12 in = 4.3666.. RT 4.37 in

Exponents, Logarithms, and Such

Exponential Numbers

10^n multiplying: add / when dividing: subtract

- $10^3 \times 10^4 = 10^7$ $10^6 / 10^4 = 10^2$
- $10^{-2} X 10^3 = 10^1$ $10^{-4} / 10^{-6} = 10^2$

2^n same rule

- $2^2 X 2^{-5} = 2^{-3}$ $2^2 / 2^{-5} = 2^7$

Logarithms - Base10 (log 0.0000 undefined)

1	0.0000	10	1.0000
2	0.3010		
3	0.4771		
4	0.6021	40	1.6021
5	0.6990		
6	0.7782		
7	0.8451	70	1.8451
8	0.9031		
9	0.9542		
10	1.0000	100	2.0000

Units of Measure and Conversions

Weight

- Short ton, lb, oz
- Metric ton, kg, g, mg, µg, ng
- Key Conversions
  - 453.592 g/lb; 2.20462 lb/kg
  - 28.3495 g/oz
  - 1 metric ton = 1000 kg =
    1.10231 short tons =
    2204.62 lb

Distance

- Mile, rod, yards, feet, inches
- Km, m, cm, mm, µm, nm
- Key Conversions
  - 1.60934 km/mi; 39.3701 in/m
  - 0.914403 m/yd; 1.09361 yd/m
  - 0.393701 in/cm
  - 2.54 cm/in; 25.4 mm/in

Units of Measure and Conversions

Area

- mile^2, acres, yd^2, ft^2, in^2
- km^2, hectare, m^2, cm^2, mm^2
- 1 Acre = 43,560 ft^2
- 640 A/mi^2
- 1 Acre = 0.404686 ha
- 1 ha = 100m X 100m =10,000 m^2
- 1 ha = 2.47105 A
- 100 ha - 1km^2

Volume

- Gallon, quart, pint, cup, Tbsp, tsp, fl oz
- Liter, dL, cL, mL, uL
- 3.78541 L/gal, 0.946353 L/qt
- 29.5735 mL/fl. oz; 14.7868 mL/Tbsp

Units of Measure and Conversions

Temperatures

- °C, °F
- °Celsius to °Fahrenheit
- F = 9/5C + 32, for instance 37°C
- F = (1.8 X 37) + 32 = 98.6°F

- °Fahrenheit to °Celsius
- C = 5/9 (F - 32), for instance -4°F
- C = 5/9 (-4 -32) = -20°C

Time

- Year, month, week, day, hour, minute, second, millisecond

Density of Water

Temperature °F/°C	Density g/cm^3
32/0	0.99987
39.2/4.0	1.00000
40/4.4	0.99999
50/10	0.99975
60/15.6	0.99907
70/21.1	0.99802
80/26.6	0.99669
90/32.2	0.99510
100/37.8	0.99318

Basic Statistical Calculations

Mean = average = sum of values/number of values

- (8+6+9+5)/4 = 28/4 = 7

Standard Deviation (SD)

- $[\Sigma(x_i - \mu)^2/n]^{1/2}$ = 1.826 as above

Relative Standard Deviation a.k.a. Coefficient of Variation =

- (SD/mean) x 100% = 26.09% as above

Correlation coefficient r

- Number between –1 and 1 measuring the strength and direction of the relation-ship between two variables (i.e., x,y)

Coefficient of determination r^2

- 1-(RSS/TSS) = number between 0 and 1 that measures how well a statis-tical model predicts an outcome

Median

- Arrange the numbers in order from smallest to largest. The number in the middle is the median odd numbers.
- For even numbers, median is the mean of the two middle numbers.

Application Rate & Gallon per Acre Calculation

Prior to Sprayer Application:

- Sprayer must be cleaned or have record of most recent cleaning
- Balance or scale used for test substance weighing must be calibration checked and appropriate for weight measured
  - Check weights bracket the weight of test substance to be measured
    - For solid test substance formulations only
  - Volume for liquid test substances made with accurate volume measure (to deliver) appropriate for volume to be measured

Application Rate & Gallon per Acre Calculation

Prior to Sprayer Application

- Individual sprayer head volume discharge using clean water must be measured in mL per measured time to provide mL per second spray rate per SOP (including reps)
- Use quality graduated cylinders (class A best) or similar and standardized/traceable stopwatch preferably with centisecond or millisecond capabilities
- Measured volume is based on calculated pass time to deliver desired rate
- Tractor speed to deliver is checked with multiple passes (propulsion gear/ engine rpm or speed control)

Plot Size and Application Rate

Experimental plots are small: for instance, 20 ft wide by 50 ft long.

Application based on lb ai/Acre or g ai/hectare.

20’ X 50’ = 1000 ft^2

1000 ft^2/43560 ft^2/A = 0.0230 A

If app rate is 1.50 lb ai/A X 0.0230A = 0.0345 lb ai X 453.592 g/lb = 15.6489 g ai to be applied to plot

Spray mixes are made up in excess (overage), often up to 25% proportionally equivalent, to assure complete delivery.

For liquid concentrates in gal/A

If applied at 4.17 lb ai/gal X 453.592 g/lb = 1891.5 g ai/gal

1891.5 g ai/gal / 3.7854 L/gal = 499.7 g ai/L

15.6489 g ai/1891.5 g ai/gal = 0.008273 gal X 3785.4 mL/gal = 31.32 mL liquid concentrate for spray mix

App rate of 20 gal/A X0.0230 A = 0.460 gal X 3785.4 mL/gal = 1741.3 mL total volume to be applied to plot

See slides 13-14 for formulas and conversions

Sprayer Calibration and Pass Time

From the example in the prior slide, with a sprayer width of 20 ft from 4 spray heads, typically 3 reps of timed volume collected provide flow determined a mean of 50.0 mL/sec per head. Individual heads must agree within a value provided in an SOP, typically within 5-10 %.

Totaling 50.0 X 4 = 200 mL per second, target pass time will be 1741.3 mL / 200 mL/sec = 8.707 seconds to deliver the desired application rate. This example plot requires only one pass. Wider plots may require multiple passes.

Tractor speed for 50 ft/8.707 sec = 5.743 ft/sec X 1 mi/5280 ft X 3600 sec/hr = 3.916 mph

Pass times are practiced and measured with a standardized timepiece with centisecond or millisecond capabilities, measured as the sprayer passes the plot ends provided by markers for the entrance and exit points.

For tractor-based sprayers, selected engine rpm and gear selection or speed control provides uniform speed for application.

Once pass time is determined, the actual application is made with time measured. Note that the sprayer is turned on before entering the plot and after leaving the plot to assure uniform application.

The actual application rate is determined as shown in formulas on the 2 next slides.

Application Rate & Gallon per Acre Calculation - Liquid Test Substance

To calculate liquid test substance application in lb ai/A =

$$\frac{\text{mL/sec calibrated X total field pass time (sec) X mL liquid test substance X ai in w/w}^{1}\text{ X density in g/mL}}{\text{Total spray vol (mL) X 453.592 g/lb X treated plot Acreage}}$$

Actual pass time must meet SOP criteria (typically +/- 5-10% of target)

To convert to g ai/hectare, multiply lb ai/A X 2.47105 A/ha X 453.592 g/lb

- 1 lb ai/A = 1.1208 kg ai/A = 1120.8 g ai/A

To calculate gallons per acre (GPA)

$$\frac{\text{mL/sec cal X sec spray time}}{\text{3785.4 mL/gal X plot Acreage}}$$

To convert to liters per hectare, multiply gal/A X 2.47105 A/ha X 3.7854 L/gal

[1]ai as decimal w/w purity – for instance, 51.2% w/w = 0.512 w/w

Application Rate & Gallon per Acre Calculation - Solid Test Substance

To calculate solid test substance application in lb ai/A

$$\frac{\text{mL/sec calibrated X total field pass time (sec) X g solid test substance X ai in w/w}^{1}}{\text{Total spray vol (mL) X 453.592 g/lb X treated plot Acreage}}$$

Actual pass time must meet SOP criteria (for instance, +/- 5-10% of target)

To convert to g ai/ha, multiply lb ai/A X 453.592 g/lb X 2.47105 A/ha

- 1 lb ai/A = 1.1208 kg ai/A = 1120.8 g ai/A

To calculate gallons per acre (GPA)

$$\frac{\text{mL/sec cal X sec spray time}}{\text{3785.4 mL/gal X plot Acreage}}$$

To convert to LPH, multiply gal/A X 2.47105 A/ha X 3.7854 L/gal

[1]ai as decimal w/w purity – for instance, 51.2% w/w = 0.512 w/w

Laboratory Tests

Used with

- Residue determinations
- Soil studies
- GLP Test, Control, Reference Substance Characterization
- Stability, Homogeneity, and Dose Prep Analyses
- Environmental and Metabolism Studies
- Specialized and Ubiquitous Other Studies

Laboratory Calculations

Analytical Tests for Residue Measurement

- Chromatography – HPLC, GC, IC, LC-MS/MS, GC/MS
- ICP, ICP-MS, wet chemistry for trace metals, unique elements in active ingredients
- Reference substances = standards
- Samples = controls & treated test system components for analysis

Analytical Tests for Residue Measurement -

Prep and dilution of standard/analyte by weight and volume

- Weight (mass) measured by calibration-checked balance
  - with appropriate accuracy
- Generally 4 or more decimal place analytical balances
- Liquid measurement – accurate/precise
  - to contain (volumetric flask)
  - to deliver (pipet)
- Concentrations used prepared by quantitative dilution
- System suitability – run pre-analysis

Analytical Tests for Residue Measurement

x = dependent variable = concentration (units)

y = independent variable (signal/area counts)

- Quantitation includes sample weights (and dilution when used)

Analytical HPLC, GC may use smaller number of standards for curve

- Often smaller range of quantitation than LC-MS-MS

Acceptance criteria for primary standards - chromatographic analyses

- r correlation coefficient – desirable values near +1 (0.9999 for instance)
- r^2 coefficient of determination – (0.9998 for instance above)

Analytical Tests for Residue Measurement
External Standard Calibration

- Standard "curve" typically linear, $y = mx+b$;
  - $x = (y-b)/m$ where y=area counts; x=concentration
  - Standard curve plots absolute value of analyte (y-axis) to concentration (x-axis)
- Higher order curve fit, $y = ax^2 + bx + c$, may be used when necessary from method validation and response factors
  - $x=[-b\pm\sqrt{(b2-4ac+4ay)}]/2a$ (positive value of x only)

Typically $\geq$6 concentrations of standards used for range of quantitation

- LLOQ, ULOQ
- LOD, LOQ

- Analysis includes standards, blanks, controls, QCs, samples, and acceptance criteria for standards and QCs
- Calculated concentration takes dilution, sample mass into account

Analytical Tests with Internal Standards (IS)

Internal standard (IS) – is added at same concentration to all standards, QCs, controls, blanks and samples, added as early in prep as possible

- Use IS for analytes
  - Analyte is in complex matrix
  - Multiple step sample preparation
- IS compound usually similar to analyte but different mass
  - Chemical analog, stable isotopic label with deuterium or C^{13} or other suitable compound
  - IS peak adequately separated from analyte; preferably eluted after analyte
  - Compatible with detector response
- Instead of calibration on absolute response of the analyte (i.e., external standard):
  - Standard (calibration) curve plots *ratio* of analyte/IS signals (y-axis) to concentration (x-axis).
- Calculated concentration takes dilution, sample mass into account.

Analytical Tests for Residue Measurement

Acceptance Criteria

- Secondary Standards (separate weighing of reference substance used as primary std)
  - Typically in range of 100+/- 2% to 100 +/- 2-20% of reference value
- QCs (recovery spikes or similar)
  - Typically in range of 100+/- 10 to 20% of expected value
- Blanks – standard prep with no added standard
- Controls – prep same as QCs and samples with no added standard

If a sample quantitated value falls above the range of quantitation, it is diluted into range.

- Dilution (i.e., 1:2, 1:10, 1:20…) provides results in range of quantitation. The inverse of the dilution factor is multiplied to provide the analyte's reported concentration.

If a sample quantitates at or below the LOQ but ≥LOD, it is reported as <LOQ. If <LOD, reported as <LOD.

Other Laboratory Tests

(Not a comprehensive list)

Quantitative

Titrations (Karl Fischer moisture content)

Residual Ash (Residue on Ignition)

Impurities (of test, control, reference substance) determinations

- Use chromatography; amounts may be semi-quantitative by area % or quantitative, w/w% or by known impurity standards (validated method).

Qualitative

- Fourier Transformed Infrared Spectroscopy (FTIR)
- Nuclear Magnetic Resonance Spectroscopy (NMR)
- Differential Scanning Calorimetry (DSC)

About Radioactivity Used in Metabolism and Environmental Studies

Radioactivity allows for i.d. of breakdown products and distribution in biological systems

The Curie (Ci) is a large amount of radioactivity.   Typically, doses of milli-, micro- or nanocuries are used experimentally.

In studies using radioisotopes, the chemical component can be analyzed with chromatography.

Beta and gamma radioactivity is typically measured by LSC.

Most studies using radioactivity use ^{14}C or ^{3}H (tritium) labels – beta emitters. Other radioisotopes such as ^{32}P may be used.

Using the concentrations and peaks determined by chromatography and radioactivity detected by LSC fractions, metabolites/breakdown products can be identified and quantified.

Units of Radioactivity for Radioactive Decay

Disintegration determined by Liquid Scintillation Counting (LSC)

- dpm – disintegrations per minute (60 seconds);
- dps – disintegrations per second

Curie - unit of radioactivity   1 Ci = 3.7×10^{10} dps from 1 gram of radium

Becquerel - unit of radioactivity

1 Bq = 1 dps   60 Bq = 1 dpm

2.22×10^6 dpm = 1 µCi

2.22×10^6 dpm X 1 min/60 sec = 3.7×10^4 dps

= 3.7×10^4 Bq = 1 µCi

1 Curie = 10^3 mCi
= 10^6 µCi
= 10^9 nCi

Dosing Calculations for In-life Studies

mg (ai)/kg BW dose - Example

300 mg ai/kg BW X 297 g BW X 1 kg/1000 g = 89.1 mg for dosage

mL dosing liquid - Example

5 mL (max dose)/kg BW X 297 g BW X 1 kg/1000 g = 1.485 mL dose vol

mg ai/mL dose liquid concentration - Example

89.1 mg ai/1.485 mL dose liquid = 60 mg/mL prep

Parting Thoughts

While all components of a GLP Study are important, I defer to the wise words of a respected peer:

- "It's all in the numbers."

Quantities are often the defining criteria, even when small, such as incidence and other criteria which are often qualitative – from the subtle to the obvious.

QA is charged with reviewing all aspects of study data to assure… "that the reported results accurately reflect the raw data of the study." Appropriate QA review of data is essential to fulfilling this requirement.

Thank you for your attention.

Links and References

US EPA GLP, 40 CFR Part 160, particularly sections 160.35 (QA), 160.130 (study conduct), and 160.185 reporting study results)

OECD SERIES ON PRINCIPLES OF GOOD LABORATORY PRACTICE AND COMPLIANCE MONITORING Number 1 OECD Principles on Good Laboratory Practice (as revised in 1997)

NIST Handbook 44 – 2018 Appendix C – General Tables of Units of Measurement

US Nuclear Regulatory Commission Library – Becquerel (Bq) https://www.nrc.gov/reading-rm/basic-ref/glossary/becquerel-bq.html

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