<u>Class Notes for Chapter 6 – Bioassay, RP & Synergy</u>

<u>Reminders</u>:

- HWK13 due Tuesday 4/15, HWK14 due Thursday 4/22
- Check webpage for HWK15 (due Tuesday 4/27)
- Quiz#3 (on NLIN = Chapter 5 only) is on Tuesday 4/20 ... 8.15am

Relative Potency

- Relative potency (ratio of two Normal or otherwise means) is a nonlinear function: need to use techniques of Chapter 5 here; Fieller-Creasy problem in depth in §6.7 (p.49ff)
- <u>Direct Assay</u> (pp. 1-8) versus <u>Indirect Assay</u> (pp. 8-13)
- <u>Direct Assay</u> examples (6.1, 6.2, and 6.3); <u>Indirect Assay</u> examples (6.4 and 6.5)
- Next topic: Assessing <u>synergy</u>/<u>antagonism</u> using two models: the Finney model and the Separate Ray model
- Example 6.1 (pp.1-4): $y = \text{sodium excretion rate (initially assumed Normal with constant variances) for two treatment groups, NORMAL (n₁ = 7) and B10AE (n₂ = 7); relative potency is estimated to be 0.426 (top of p.2).$
- To get a CI (Wald or Likelihood), use NLIN approach with mean E(Y) = μ₂ for NORMAL group and mean E(Y) = μ₁ = ρμ₂ for B10AE group (using dummy variables in Eqns. 6.2 and 6.3)
- So mean = $\rho \mu_2 * B10TRT + \mu_2 * NORTRT$ (model function)
- PROC NLIN on p.3 assumes Normality and constant variances
- PROC NLIN gives RP 95% WCI (-0.0177,0.8697): we are thus 95% confident that the true RP (of B10TRT to NORMAL) lies between -0.02 and 0.87. Since one (1) is not in the CI, we're confident that they're not equally potent (B10TRT is less potent).
- Left endpoint of CI and right skewness in p.2 plot makes us doubt our assumptions here let's return to the theory. If Y₁ = ρY₂, then log(Y₁) = log(ρ) + log(Y₂). Y₁ is conc. of substance 1, and Y₂ is conc. of substance 2. Now, let Z₁ = log(Y₁), and

assume $Z_1 \sim \text{Normal}(v_1, \sigma^2)$; similarly for substance 2. Plots of Z's given on p.3 look more <u>Normal with constant variance</u>.

- New mean relationship is given in Eqn. 6.6 and fit in the NLIN which produces Output 6.1c. Now, 95% WCI for true RP is (0.0803,0.6843). It is good to see interval doesn't go into negative values (impossible). The 95% PLCI is (0.1735,0.8424), and this is the one we should use since Likelihood method is best.
- Example 6.2 ratio of two independent Poisson means (since these are COUNT data) using NLMIXED procedure (p.5). RP of SOAP ($n_1 = 8$) to CONTROL ($n_2 = 6$) is estimated to be 0.6028 and Wald TS testing equal potency is on the top line of p.6. SAS implies this TS ~ t_{14} (most statisticians would argue ~ t_{12}). Likelihood test REDUCED model is fit after line 7 on p.6 (is this right?). Results aren't shown but reported: $\chi_1^2 = 28.2$, p < 0.0001. What is our conclusion here?
- Example 6.3 Y = prostate size for $n_1 = 5$ CONTROL and $n_2 = 5$ ESTRIADIOL animals. Plot is given on p.6: data look Normal (symmetric) but variance is not constant – see NLIN residual plot on p.7. Let's model variances too! If $Y_1 = \rho Y_2$, then $\mu_1 = \rho \mu_2$ and $\sigma_1^2 = \rho^2 \sigma_2^2$. This is kind of like the Seefeldt example (5.8) from last class. See NLMIXED program on p.7 (why can we **not** use NLIN here?); the 95% WCI for RP is (1.84, 5.00). Profile likelihood curve is on p.8 with cut-lines at 90% (bottom line), 95% (middle) and 99% (top). From 95% cut line, we see 95% PLCI is (2.19, 5.34). <u>Conclusion</u>: we're 95% confident that Estriadiol is at least 2.19 times and as much as 5.34 times as potent as Control. Since one (1) is not in the PLCI, we conclude that Estriadiol is significantly more potent than Control.
- For **Indirect Assays**, we cannot measure amounts directly, but must make inferences indirectly. Thus, we'll fit dose-response curves such as the Binary/Binomial logistic or other nonlinear model function. When we do, we usually assess RP (relative potency) by the ratio of the LD₅₀'s for the two treatments.

- Example 6.4 compares two peptides, Neurotensin (N) and Somatostatin (S) using two Binary logistic models. Aside: note the chosen design here – start with either 0.01 and then multiply by 10^k or start with 0.03 and then multiply by 10^k. Looking at the graph on p.9, looks like the doses don't go high enough.
- First step: We have to decide which scale to use jump forward to Box-Cox transformation Eqn. 6.14 on p.21: when θ₆ is near 0 (as is the case here), then use log-dose.
- Now look at the program on p.10, and write down the explicit formula for π (success probability).
- The first NLMIXED here has unequal slope parameters (θ_3) and the second one (Reduced model) has a common slope: -2LL's are given in table on p.11. Here, we retain the assumption of common slopes (p=0.1213).
- Then, RP is estimated to be 5.66: which peptide is more potent?
- As to CI's look at Reduced model output (Output 6.4) on p.10: 95% WCI, (-1.89, 13.2) looks weird. Why?
- Profile likelihood plot on p.11. Really good eyesight confirms that 95% PLCI for ρ is (1.59, 19.59). Interpretation is at bottom of the page. Consequence/ramification are ...?
- Example 6.5 on p.12 gives a Normal example with a modification of the MM2 model function in Eqn. 6.9; here, θ₁ is the upper asymptote but what is θ₂? Testing for common upper asymptotes programs on top of p.13, and here we do the Full-and-Reduced F test on bottom of p.12 (accept same upper asymptote).
- Reduced model is in Output 6.5, and RP is estimated to be 0.0420 ≈ 0.04 = 1/25, so standard insulin is approximately 25 times more potent than the A1-B29 insulin variety.

Synergy/Antagonism/Interaction

- We can assess interaction (synergy or antagonism) using either of the Finney models or the SR model
- The Finney models first combine two x's (e.g., doses of two drugs) in the effective dose formula (Equation 6.10), and then relates this effective dose (denoted 'z') to the response variable using either Equation 6.11 or 6.12 or some variant of these
- θ_5 is the key (so-called coefficient of synergy) parameter, with
 - $\theta_5 > 0$ indicating synergy
 - $\theta_5 < 0$ indicating antagonism
 - $\theta_5 = 0$ indicating independent action
- As noted last class, Equation 6.12 is the binary logistic model function using the **log-dose scale** in practice, one needs to determine which exact scale to use and modify accordingly
- Example 6.6. Gerig 2 phenolic acids (ferulic and vanilic acids) in 3 chambers (blocks). Chosen design in graph on p.16 (six support points, only one of which is an 'interior point'). NLIN output on p.16 indicates significant **antagonism**, but Likelihood (Full and Reduced) test gives only marginal proof: p-value = 0.0254. Clearly need a better study! See the **isobole** on p.16.
- Example 6.7. Upjohn drugs A and B binomial example design in graph on p.18 (plus additional support points). n_k mice given a given combination of A and B, and y_k = number that die is counted; log-scale is indicated (output not shown). These data indicate significant **synergy** between drugs A and B (p.18).
- Example 6.8. Carter's ethanol and chloral hydrate binomial study; checkerboard design on p.19. Maybe a "<u>Ray Design</u>" would be better. Evidence here of synergy (p = 0.0151).
- Example 6.9. Machado & Robinson. Y = RT activity (counts). Drugs are AZT and ddI. Ray design on p.20 with 3 interior rays Normal fit produces conclusion of independent action and the residual plot on p.20 – looks ☺ Refit using <u>Poisson</u> distribution

and **<u>normal w/modeled variance</u>** – got similar results; former is on p.21. Conclude significant synergy between these two drugs.

- Example 6.10. Chou and Talalay example shows the need for the Box-Cox scale parameter (θ_6) since it's estimate is neither zero (log-dose) nor one (dose) here. Also, response variable here is a fraction, so we take logit transformation to (hopefully) achieve Normality. Then, we observe significant synergy.
- Sometimes the <u>Finney models</u> are not rich enough and we need a larger model such as the <u>Separate Ray (SR) model</u>. The SR model allows for e.g. synergy for one ray, independent action for another, and antagonism for a third. Note for example that for the Finney model to fit, the slopes must be equal and the LD₅₀'s must line up on an **isobole** as those given on p.16 or p.18 the point being that it is a rather 'narrow' or restrictive model. (That said, the Finney model does fit in some cases).
- Lots of notation in the SR model, but the big picture is in the graph on p.24. Point C is the LD₅₀ for Drug B and point E is LD₅₀ for Drug A. Rays 3 ... J ... R are interior rays corresponding to different proportions of drugs A and B (with "slopes" c_k in Equation 6.15). For Ray J, if the LD₅₀ is at the point D, then the compounds exhibit independent action. If it is closer to the origin, we have synergy, and further from the origin is associated with antagonism). A measure of the actual LD₅₀ to the one expected under independent action is the combination index (denoted κ_r) for each interior ray. The SR model simultaneously fits separate logistic (or otherwise) curves along each of the rays, and calculates the κ_r's.

$\kappa_r = 1 \rightarrow$ independent action	n
$\kappa_{\rm r} < 1 \rightarrow {\rm synergy}$	
$\kappa_{\rm r} > 1 \rightarrow$ antagonism	

- It can be shown that if all the slope parameters (the θ₃'s) are equal and the κ_r's follow a specific algebraic relation, then <u>the</u> SR reduces to the Finney model. Implications for nesting!
- Example 6.11. Martin. On p.26, just one interior ray. Six design points on the interior ray, and 5 on the two exterior rays. Point A is the LD₅₀ for Deguelin, point B is LD₅₀ for Rotenone, point C is the intersection with interior ray, and point F (filled circle) is the actual LD₅₀ along the interior ray, so κ₃ = 0.6615. Note that Output 6.10a (SR model) here is better than 6.10b (equal slopes) for these data (p = 0.0042). Wald test of H₀: κ = 1 is on p.27 better yet, using the program near the bottom of p.27, likelihood –2ΔLL test gives χ₁² = 14.3, p = 0.0002. Finally, since RP estimate is ρ̂ = 2.6405, the interior ray corresponds to the effective fraction f = 0.6053 (via Equation 6.19).
- Example 6.12. Additional Binomial examples with one interior ray here. Hewlett and Plackett DDT and γ -BHC again. Output 6.11a shows that log-dose and dose scales are wrong for these data see Equations 6.13 and 6.14 (p.21): use this new scale for these data. Then, can accept equal slopes ($\chi_2^2 = 0.8$), but not independent action synergy detected here too; $\hat{\kappa}_3 = 0.4555$.
- Example 6.13. Shelton data: response variable here is a fraction, and transformed to Normality with the logit transformation one interior ray here. Cannot accept common slopes see Full & Reduced F on p.30, so the Finney model will not fit these data. Synergy detect here κ₃ = 0.4286 and c = ¼ → f = 0.2605, which may be too low. See Equation 6.17 on p.23. This example points out that we need a good estimate of ρ = relative potency before we choose the slope of the ray(s), c.
- Example 6.9 continued. Finney model even with the Poisson distribution doesn't fit well residual plot on p.31 looks wavy. Separate Ray model fits better see p.32. This dataset has 3 interior rays with slopes c = 10, 5, and 1. Synergy is detected

along each ray, and we accept a common combination index; test that it equals 1 is rejected ($\chi_1^2 = 218.9$, p < 0.0001). Relative potency estimate is such that these interior rays correspond to the effective fractions f = 0.1588, 0.2741, and 0.6537.

• Example 6.14 (not 6.15). Goldin's cancer example – three interior rays with slopes c = 7.5, 1, and 1/7.5. Graph is given on p.33. We see independent action along first ray, marginal result along the central ray, and strong synergy along gentle-sloped ray. Combination indices can be related to effective fractions as in plot on p.35.

<u>Next/Last Class</u>: return to Chapter 7 and analyzing nonlinear longitudinal data using NLME/NLMIXED