Class Notes for Chapter 6

Class One

- Relative potency (ratio of two [Normal or otherwise] means) is a nonlinear model – need to use techniques of Chapter 5 here
- Direct Assay (pp. 1-9) versus Indirect Assay (pp. 9-16)
- Direct Assay examples (6.1, 6.2, and 6.3); Indirect Assay examples (6.4 and 6.5)
- Example 6.1 – ratio of two independent Poisson means (since these are COUNT data) using NLMIXED procedure (p.3). 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 bottom of p.3. SAS implies this TS ~ t_{14} (some would argue ~ t_{12} or ~z). Likelihood test REDUCED model is fit on p.4 (is this right?): $\chi_1^2 = 28.2$, $p < 0.0001$. What is our conclusion here?
- Example 6.2 (pp.4-6): y = sodium excretion rate SER for two treatment groups, NORMAL ($n_1 = 7$) and B10AE ($n_2 = 7$) – since original data are R skewed, use log-transformed data
- If $Y_2 = \theta_2 Y_1$, then $\log(Y_2) = \log(\theta_2) + \log(Y_1)$. Y_1 is conc. of substance 1, etc. Now, let $Z_1 = \log(Y_1)$, and assume $Z_1 \sim$ Normal(ν_1,σ^2), etc. for substance 2. Plots of the Z's given look more Normal with constant variance.
- New mean relationship is in Eqn. 6.6 and fit in the NLIN which produces Output 6.2a. Now, 95% WCI for true RP is suspect, so we do the Likelihood test, and get p = 0.0211.
- Example 6.3 – Y = prostate size for $n_1 = 5$ CONTROL and $n_2 = 5$ ESTRADIOL animals. Plot on p.7: data look Normal (symmetric) but variance is not constant. Let's model variances too! If $Y_2 = \theta_2 Y_1$, then $\mu_2 = \theta_2 \mu_1$ and $\sigma_2^2 = \theta_2^2 \sigma_1^2$. This is kind of like the Seefeldt example (5.8) from last class. See NLMIXED program on pp.7-8 – why can we *not* use NLIN here? 95% WCI for RP is

(1.84,5.00). Profile likelihood curve is on p.9 with cut-lines at 90% (bottom line), 95% (middle) and 99% (top). From 95% cut line and really good eyes, 95% PLCI is (2.19,5.34). Conclusion: we're 95% confident that Estradiol is at least 2.19 times as potent as Control; since 1 is not in the PLCI, Estradiol is significantly more potent than Control.

Class Two:

- **For Indirect Assays, we cannot measure amounts directly, but must make inferences indirectly. We'll fit dose-response curves such as the Binary Logistic or other nonlinear model function. When we do, we usually assess RP (relative potency) by the ratio of the LD_{50}'s for the two treatments.**
- **Example 6.4 compares two peptides, Neurotensin (N) and Somatostatin (S) using Binary Logistic models. Note the chosen design here: either 0.01 and then multiplied by 10^k or 0.03 and then multiplied by 10^k. Looking at the graph on p.10, looks like the doses don't go high enough.**
- **First step: Had to decide which scale to use – jump forward to Box-Cox transformation Eqn. 6.14 on p.27: when θ_6 is near 0 (as is the case here), then use log-dose.**
- **Now look at the program on p.11, 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 one) has a common slope: -2LL's are given in the outputs. Here, we retain the assumption of common slopes (test stat: $\chi_1^2 = 2.4$, p = 0.1213).**
- **Then, RP is estimated to be 5.6639**
- **Which peptide is more potent?**
- **As to CI's look at Reduced model output (Output 6.4b) on p.12: 95% WCI, (-1.89,13.2) looks weird. How so?**

- **Profile likelihood plot on top of p.13. Really good eyesight confirms that 95% PLCI for ρ is (1.59,19.59). Interpretation is after the graph. Consequence/ramification are …?**
- **Example 6.5 on p.13 gives a Normal example with the modified MM2 model function in Eqn. 6.9, where θ_1 is the upper asymptote but what is θ_2 here? Testing for common upper asymptotes – programs on pp.14-15, and here we do the Full-and-Reduced F test bottom of p.15 (accept same upper asymptote).**
- **Reduced model is in Output 6.5c, and RP is estimated to be ≈24, so standard insulin is approximately 24 times more potent than the A1-B29 insulin variety.**

Class Three:

- **We can assess interaction (synergy or antagonism) using either one of the Finney models or the SR model**
- **The Finney models combine two x's (e.g., doses of two drugs) in the effective dose formula (Equation 6.10) first, and then relates this z (effective dose) 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 vanillic acids) in 3 chambers (blocks). Chosen design in graph on p.19 (six support points, only one of which is an interior point). NLIN output on p.20 indicates significant antagonism, but Likelihood**

(Full and Reduced test) gives marginal proof: p-value = 0.0254. Clearly need a better study! See the isobole on p.19.

- Example 6.7. Upjohn drugs A and B binomial example – design in graph on p.22 (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.23).
- Example 6.8. Carter ethanol and chloral hydrate binomial; checkerboard design on p.24. Maybe a "Ray Design" 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.25 with 3 interior rays. Normal fit produces conclusion of independent action and the residual plot on p.25 – Yikes! Refit using Poisson distribution and modelling variance – got similar results, so former is on p.26. 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 its 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.

Class Four

- Sometimes the Finney models are not rich enough and we need a larger model such as the Separate Ray (SR) model. The SR model allows for e.g. synergy for one ray, independent action for another, and antagonism for yet a third. Note for example that the for the Finney model to fit, the slopes must be equal and the LD_{50}'s must line up on an isobole as on p.19 or

p.22 – the point being that it is a rather 'narrow' or restrictive model (that said, it does fit in many cases).

- Lots of notation in the SR model, but the big picture is graph on p.29. Point C is the LD_{50} for Drug B and point E is LD_{50} 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 LD_{50} is at the point D, then we have independent action. If it's closer to the origin, we have synergy (further from the origin → antagonism). A measure of the actual LD_{50} to the one expected under independent action is the combination index (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.

$\kappa_r = 1$ → independent action
$\kappa_r < 1$ → synergy
$\kappa_r > 1$ → antagonism

- It can be shown that if all the slope parameters (θ_3's) are equal and the κ_r's follow a specific algebraic relation, then the SR reduces to the Finney model.
- Example 6.11. Martin. On pp.30-31, just one interior ray. Six design points on the interior ray, and 5 on the two exterior rays. Point A is the LD_{50} for Deguelin, point B is LD_{50} for Rotenone, point C is intersection with interior ray, and point F (filled circle) is the actual LD_{50} along the interior ray, so $\hat{\kappa}_3 = 0.6615$. Note that Output 6.10a here is better than 6.10b (equal slopes) for these data (p = 0.0042). Wald test of H_0: $\kappa = 1$ is on p.31 – better yet, using the program near the bottom of p.32, likelihood $-2\Delta LL$ test gives $\chi_1^2 = 14.3$, p = 0.0002. Finally, since RP estimate is $\hat{\rho} = 2.6405$, the interior ray corresponds to the effective fraction $\hat{f} = 0.6053$ (Equation 6.19) on p.29.

- **Example 6.12.** Additional Binomial examples with one interior ray. 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. Stay on 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.35, so the Finney model will not fit these data. Synergy detect here $\hat{\kappa}_3 = 0.4286$ and c = ¼ → $\hat{f} = 0.2605$, which may be too low. See Equation 6.19 on p.29. 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.36 looks possibly wavy. Separate Ray model (with Poisson dist.) fits better – see p.37. 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.** Goldin cancer example – three interior rays with slopes c = 7.5, 1, and 1/7.5. Graph on p.38. Independent action along first ray, marginal synergy along the central ray, and strong synergy along gentle-sloped ray. Combination indices can be related to effective fractions as in plot on p.40.