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# Fitting Binomial, Quasi – Binomial, Poisson and Quasi - Poisson Models in Analyzing In Vivo Micronucleus Assay Data

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## ABSTRACT

Micronucleus assay is an important analysis for testing the genotoxicity of a compound thus, a careful analysis should be done. The main focus of this study was to perform the recommendations of Hothorn and Gerhard (2009) on analyzing the number and proportion of micronucleus polychromatic erythrocytes for a given number of polychromatic erythrocytes. The compound TO1 was evaluated using the Binomial, Poisson, quasi-Binomial and quasi-Poisson models. Results showed that most doses of TO1 had no significant effect on the proportion of micronucleus. The result of quasi-Binomial and quasi-Poisson model revealed that most of the inferences agree with the results of the traditional models. However, some inferences can lead to a false positive decision if over-dispersion was not taken into account. The analysis also showed that TO1 was found to be a negative compound.

**Key Words:** binomial, Poisson, quasi-Binomial, quasi-Poisson, over-dispersion

## INTRODUCTION

Micronucleus test has been recommended as part of the 'minimal package' for testing new pharmaceutical products by the Committee of Proprietory Medical Products (CPMP) of the European Economic Community (EEC). Hothorn and Gerhard (HG) (2009) suggested a statistical methodology on analyzing in vivo micronucleus assay. The data from in vivo are frequency of micronucleated erythrocytes cells (MN) per a particular number of polychromatic erythrocytes (PCE).

In the in vivo micronucleus assay, the individual animals are the experimental units which are randomized and treated. Therefore, the variability between the animals should be taken into account (HG, 2009). HG emphasized that pooling the number of MN in each animal disregards the variability between experimental units. To address this problem, they proposed to model the between-animal variability using quasi-Binomial and quasi-Poisson models.

Another statistical aspect is the use of confidence interval instead of p-value. HG also proposed the type of inference, either to identify an increasing dose-related trend, possibly with

downturn effects at high doses (Bretz and Horthon, 2003). Finally, HG proposed a proof of safety approach to determine if the compound is not genotoxic.

In this study, different models were evaluated - Binomial, quasi- Binomial, Poisson and quasi-Poisson. The quasi models were used to handle overdispersed data which occur if the variability between experimental units are not taken into account. The purpose of this study was to apply the methodology based solely on the recommendations of HG by fitting different models. Likewise, the threshold tolerability values were adopted from the same study. Application of the techniques was performed independently for each harvesting time.

## MATERIALS AND METHODS

Data source procedure. This study used data from a genotoxicity testing experiment of compound TO1 (code name for confidentiality purposes). The data came from an assay with compound TO1 with different harvesting times (48 hrs. and 72 hrs.). Male mice were randomly allocated into three different dose levels of testing groups – low, medium, high and a concurrent negative, and optionally, a positive control group. Five to six mice were selected randomly in each group. In total, 20 male mice in each harvesting time was used and analyzed independently. Each mouse in the vehicle control was given demineralized water while each mouse in the remaining groups was treated with a certain dose of TO1. Next, blood samples were obtained for each mouse based on the harvesting time after dosing specified in the protocol. Micronucleus frequencies were determined for each animal by scoring 20,000 polychromatic erythrocytes (PCEs) and the micronucleus occurrence per 20,000 PCEs was recorded (Table 1).

**Table 1.** Dosing scheme for the micronucleus assay

| Dose Group | No. of Rat | Harvest Time (h) | Frequency of Dosing |
|---|---|---|---|
| Vehicle Control | 5 | 48, 72 | Single Dose |
| Low | 5 | 48, 72 | |
| Medium | 5 | 48, 72 | |
| High | 5 | 48, 72 | |
| Positive Control | 5 | 48 | |

Statistical analysis. The proportion of micronucleus per polychromatic erythrocytes (MNPCE) and the number of MNPCE were analyzed by logistic and log-linear models respectively. The goodness-of-fit of the model was checked with the residual deviance test. If over-dispersion exists quasi-Binomial and quasi-Poisson models were fitted. Cochran-Armitage trend test and Williams-type contrast were used to verify the dose-response trend.

For the proof of hazard approach, the following hypothesis was tested:

$$H_o: \frac{\pi_{dose}}{\pi_{control}} \leq 1 \; harmless \quad (1)$$

$$H_a: \frac{\pi_{dose}}{\pi_{control}} > 1 \; harmful \quad (2)$$

In this study, the harmlessness of the dose was concluded if the p-value of the dose vs. the control group was greater than $\alpha$ (p-value>0.05), otherwise the dose was harmful. Lastly, the proof of safety tested the following hypothesis:

$$H_o: \frac{\pi_{dose}}{\pi_{control}} \geq 3 \; harmful \quad (3)$$

$$H_a: \frac{\pi_{dose}}{\pi_{control}} < 3 \; harmless \quad (4)$$

The harmlessness of the dose was concluded if the upper limit of the relative risk (RR) was less than the three-fold threshold of tolerability.

Statistical software. All statistical procedures were implemented in R. All analyses were done at 5% level of significance.

## RESULTS AND DISCUSSION

As shown in Table 2, the estimates for the binomial model of TO1 with harvesting time of 48 hours showed that medium dose and intercept (i.e. control group) had significant effect on the proportion of micronucleus per PCE. The ratio of residual deviance and its degrees of freedom yields 1.65 which indicates over-dispersion. To account for this over-dispersion, a quasi-Binomial model was fitted. Results show there was a difference on the result of quasi-Binomial wherein medium dose had no significant effect.

Based on the Poisson model, medium dose and intercept (i.e. control group) had significant effect on the number of micronucleus. The ratio of residual deviance to its degrees of freedom is 1.66. Quasi-Poisson model revealed that intercept alone was significant and that the medium group was not significant in contrast to the result from the Poisson model.

**Table 2.** Micronucleus Assay with TO1 (48 hrs)

| | Estimate | Binomial | | Quasi-Binomial | | Estimate | Poisson | | Quasi-Poisson | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | S.E. | Sig. | S.E. | Sig. | | S.E. | Sig. | S.E. | Sig. |
| Intercept | -6.210 | 0.071 | <0.0001 | 0.092 | <0.000 | 3.694 | 0.071 | <0.000 | 0.092 | <0.000 |
| Low | 0.005 | 0.100 | 0.960 | 0.130 | 0.970 | 0.005 | 0.100 | 0.960 | 0.130 | 0.970 |
| Medium | -0.260 | 0.107 | 0.015 | 0.139 | 0.081 | -0.260 | 0.107 | 0.015 | 0.139 | 0.081 |
| High | -0.078 | 0.102 | 0.446 | 0.133 | 0.567 | -0.078 | 0.102 | 0.446 | 0.133 | 0.567 |
| Null deviance | | 34.23 on 19 df | | | | 34.489 on 19 df | | | | |
| Residual deviance | | 26.440 on 16 df | | | | 26.492 on 16 df | | | | |
| Dispersion parameter | | 3.570 | | | | 5.530 | | | | |

Table 3 presents the binomial estimates of TO1 compound with harvesting time of 72 hours. Results show that dose groups of TO1 with harvesting time of 72 hours had no significant effect on the proportion of micronuclei. Considering the ratio of residual deviance to its degrees of freedom, the value of 2.1 indicates over-dispersion on the binomial model. Consequently, a quasi-Binomial was fitted to account for the over-dispersion which gives the same inference as the binomial model. Same findings were observed from using the Poisson and quasi-Poisson models.

### Dose to Control Group Comparison

Looking at the p-value of the test on TO1 with different harvesting time (Tables 4 and 5), the comparison of each dose to control group was not significant. Considering the lower limit of RR, the dose group had no significant increase in the proportion of micronucleus compared to the control group since the lower limit of the tests did not exceed the value of 1 (i.e. hypothesized value of relative risk). The result can classify TO1 as a negative compound. Same results were observed from quasi binomial model and evidently it gives lower RR estimates.

**Table 3.** Micronucleus Assay with TO1 (72 hrs)

| | Estimate | Binomial | | Quasi-Binomial | | Estimate | Poisson | | Quasi-Poisson | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | S.E. | Sig. | S.E. | Sig. | | S.E. | Sig. | S.E. | Sig. |
| Intercept | -6.314 | 0.074 | <0.0001 | 0.108 | <0.0001 | 3.589 | 0.074 | <0.0001 | 0.108 | <0.0001 |
| Low | -0.155 | 0.117 | 0.184 | 0.169 | 0.373 | -0.155 | 0.117 | 0.183 | 0.169 | 0.373 |
| Medium | -0.099 | 0.108 | 0.361 | 0.156 | 0.537 | -0.099 | 0.108 | 0.360 | 0.156 | 0.537 |
| High | -0.195 | 0.111 | 0.079 | 0.160 | 0.243 | -0.195 | 0.111 | 0.079 | 0.160 | 0.243 |
| Null deviance | | 34.961 on 18 df | | | | 35.017 on 18 df | | | | |
| Residual deviance | | 31.488 on 15 df | | | | 31.538 on 15 df | | | | |
| Dispersion parameter | | 2.1 | | | | 5.530 | | | | |

For the Poisson model, the p-value showed that there was no significant increase in the number of micronucleus in different dose compared to control group. Likewise, the lower RR revealed no statistically significant difference on micronucleus. Both Poisson and quasi-Poisson procedure lead to the same inference.

**Table 4.** Dose to Control Group Comparison and Relative Risk of TO1 (48 hours)

| | | Traditional | | | Quasi | |
|---|---|---|---|---|---|---|
| Comparison | RR | p-value | Lower RR | Upper RR | p-value | Lower RR |
| Low – Control | 1.0050 | 0.7390 | 0.8177 | 1.2352 | 0.744 | 0.7683 |
| Medium – Control | 0.7711 | 1.0000 | 0.6181 | 0.9622 | 0.998 | 0.5781 |
| High – Control | 0.9254 | 0.9430 | 0.7497 | 1.1424 | 0.916 | 0.7035 |

RR- Relative Risk

**Table 5.** Dose to Control Group Comparison and Relative Risk of TO1 (72 hours)

| | | Traditional | | | Quasi | |
|---|---|---|---|---|---|---|
| Comparison | RR | p-value | Lower RR | Upper RR | p-value | Lower RR |
| Low – Control | 0.8564 | 0.9891 | 0.6723 | 1.0908 | 0.9645 | 0.6036 |
| Medium –Control | 0.9061 | 0.9641 | 0.7244 | 1.1333 | 0.9287 | 0.6557 |
| High – Control | 0.8232 | 0.9975 | 0.6543 | 1.0357 | 0.9845 | 0.5907 |

RR- Relative Risk

**Trend Test**

Trend test was performed to determine if the increase in dose of the compound also increases the proportion of micronucleus, i.e. increasing trend. The result revealed no significant trend for TO1 with different harvesting time yielded a p-value of 0.7461 and 0.6435, respectively. Williams contrast indicated that TO1 with different harvesting time had no increasing trend on the proportion of micronucleus since all the group comparisons were not significant. The lower relative risk supports this inference since the values were not significantly greater than one.

For the Poisson model, Table 6 illustrated the result of Williams test for TO1 with 48 hours harvesting time. Results showed no significant p-values that indicates no increasing trend. The lower relative risk supports this result since the values were less than one. Moreover, the analysis for TO1 with 72 hours of harvesting time revealed no significant increasing trend on the dose groups. The relative risk showed this inference together with the p-value.

**Table 6**. Williams Type contrast for Relative Risk of TO1

| Comparison | TO1 (48 hours) | | | TO1 (72 hours) | | |
|---|---|---|---|---|---|---|
| | RR | Lower RR | P-value | RR | Lower RR | P-value |
| C 1: High vs. Control | 0.7607 | 0.5730 | 0.9850 | 0.9061 | 0.6739 | 0.8320 |
| C 2: Medium and High vs. Control | 0.8684 | 0.6835 | 0.9230 | 0.8836 | 0.6810 | 0.8930 |
| C 3: All dose vs. Control | 0.8830 | 0.7045 | 0.9120 | 0.8616 | 0.6758 | 0.9350 |

**Proof of Hazard vs. Proof of Safety**

Binomial model was used for the proof of hazard and safety approach. For the proof of hazard, the p-value of dose groups of TO1 (see Tables 4 and 5) were not significant which means, doses of TO1 were harmless. On the other hand, on the proof of safety, upper relative risk was used because the interest was the increasing proportion of micronuclei. The compound TO1 can be concluded to be harmless since the upper relative risks in all doses were less than the threshold value.

In the case of Poisson model, the compound can be identified to be harmless since all the p-values of dose group with different harvesting time were not significant. Moreover, looking at the lower relative risks of the dose group compared to control, the values were less than one.

The test for the proof of safety was shown on Tables 4 and 5. The compound can be concluded to be harmless since the upper relative risk on having micronuclei in all doses were less than 3. This result matched to the previous tests where TO1 was harmless.

Findings revealed that estimates of binomial, Poisson, quasi-Binomial and quasi-Poisson were almost the same. This illustrates the findings of Hothorn and Gerhard that if the number of MNPCE observed in numerous polychromatic cells were too small the Poisson distribution approximates the binomial distribution. Standard errors were deflated when over-dispersion was not taken into account. The between- animal variability should be taken into account because if this was ignored, a false positive decision (i.e. harmless dose identified as harmful) may be concluded. This was observed in TO1 with harvesting time of 48 hours wherein binomial models have contradicting result with the quasi-Binomial model. In making inference, this can lead to a false positive decision (i.e. harmless dose identified as harmful) or Type I error. HG also recommended to use a number of experimental units with greater than five replicates to fit quasi-likelihood models. It is also recommended to perform the same analysis adjusted for other covariates. Lastly, a toxicologist should also be consulted to determine the harmlessness of the compound.

## REFERENCES

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