toxics

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*Article*

# Supplementary Material: Toxicological Effects of Roundup® on *Drosophila melanogaster* Reproduction

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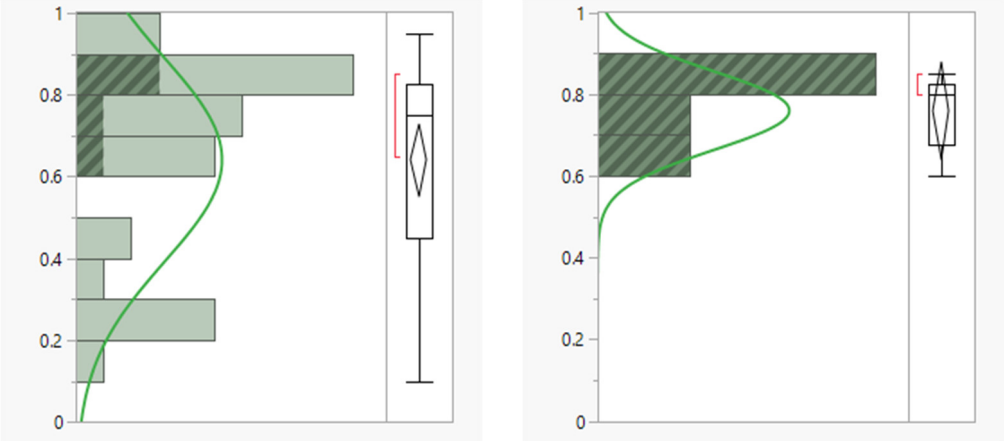
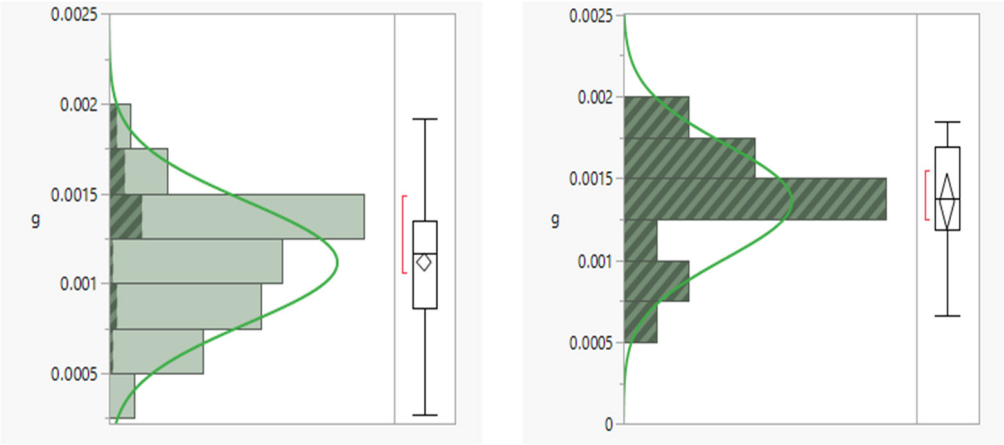
**Table S1.** Test for normality (goodness of fit test, W and $p < W$), skewness, and kurtosis for all three dependent variables based on the whole data set (overall) and each of the three exposure treatments (unexposed controls, exposed to Roundup® Super Concentrate, and exposed to Roundup® Ready to Use). Bold indicates significant $p$-values < 0.0031.

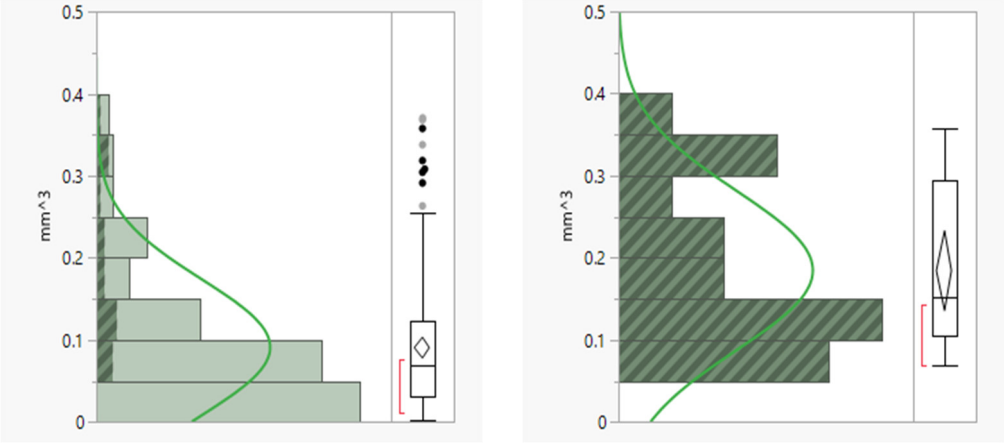
| Exposure Treatment | Test of Normality | Test Statistic | Survival (Proportion Per Vial) | Body Weight (mg) | Ovary Volume (nL) | Oocytes (Number) |
|---|---|---|---|---|---|---|
| Overall | goodness of fit to normal distribution | W | 0.874 | 0.563 | 0.355 | 0.392 |
| | | *p* | 0.0012 | <0.0001 | <0.0001 | <0.0001 |
| | Skewness | | −0.807 | −0.181 | 1.65 | 1.2 |
| | Kurtosis | | −0.626 | −0.509 | 2.97 | 1.73 |
| Controls | goodness of fit to normal distribution | W | 0.859 | 0.935 | 0.893 | 0.942 |
| | | *p* | 0.223 | 0.238 | 0.0426 | 0.31 |
| | skewness | | −1.518 | −0.621 | 0.475 | −0.128 |
| | Kurtosis | | 2.608 | −0.381 | −1.33 | −1.03 |
| Roundup® Super Concentrate | goodness of fit to normal distribution | W | 0.856 | 0.972 | 0.898 | 0.948 |
| | | *p* | 0.0856 | 0.0675 | <0.0001 | 0.0041 |
| | skewness | | −0.605 | −0.402 | 1.189 | 0.769 |
| | Kurtosis | | −1.270 | −0.398 | 1.25 | 0.337 |
| Roundup® Ready to Use | goodness of fit to normal distribution | W | 0.834 | 0.98 | 0.804 | 0.808 |
| | | *p* | 0.0038 | 0.206 | <0.0001 | <0.0001 |
| | skewness | | −0.466 | −0.211 | 2.07 | 2.12 |
| | Kurtosis | | −1.598 | −0.707 | 5.87 | 7.92 |



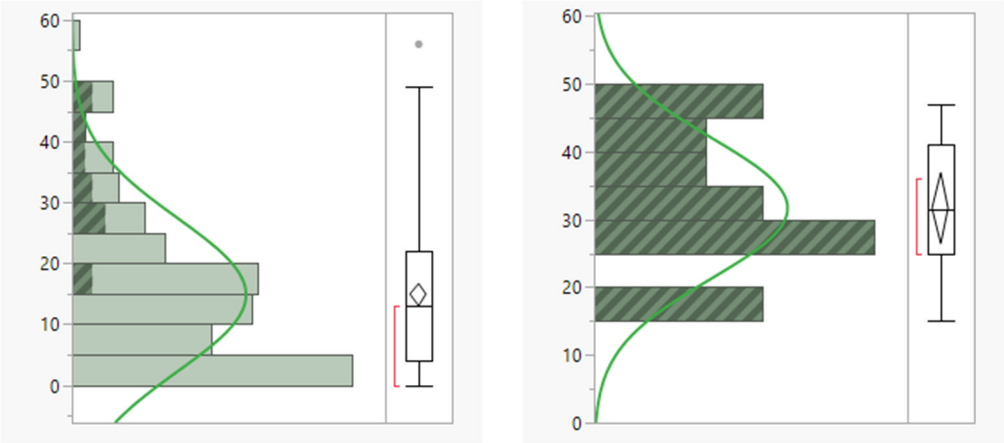

(**a**) Survival




(**b**) Body Weight

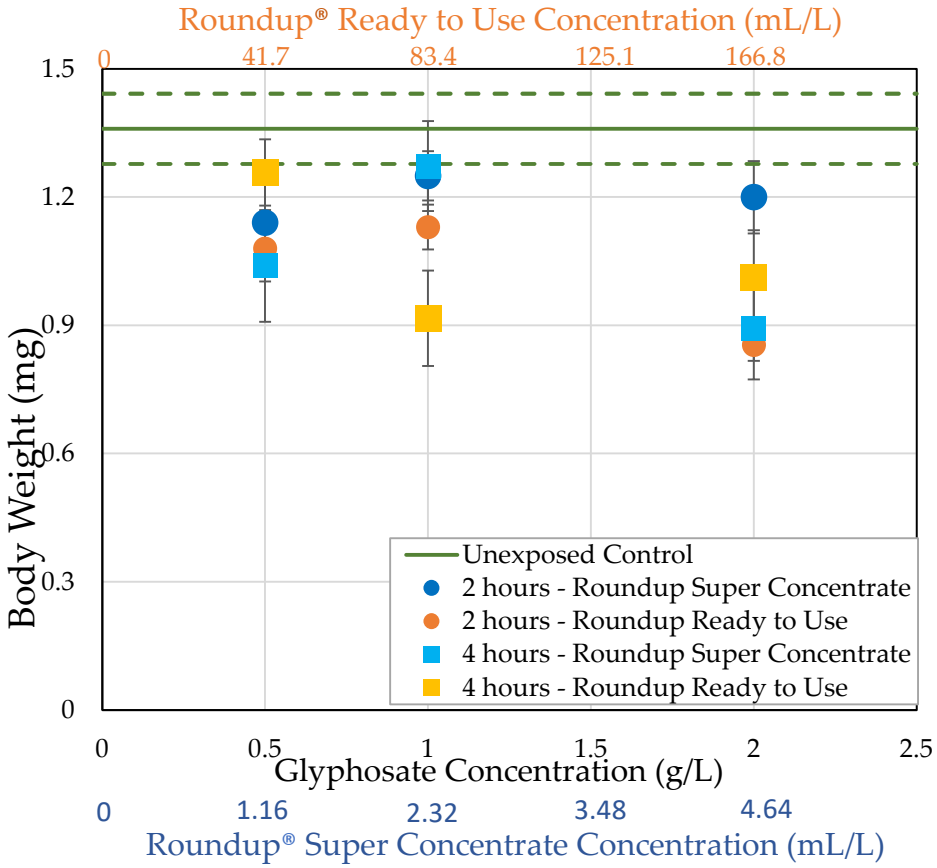



(**c**) Ovary Volume

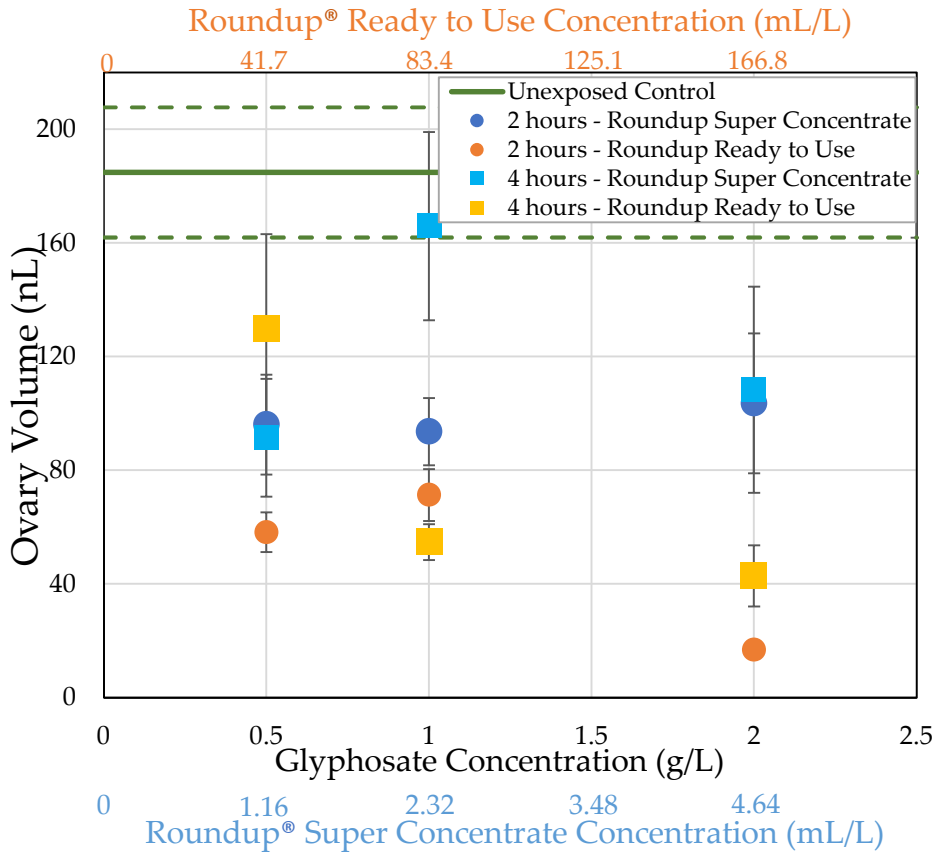



(**d**) Number of Oocytes

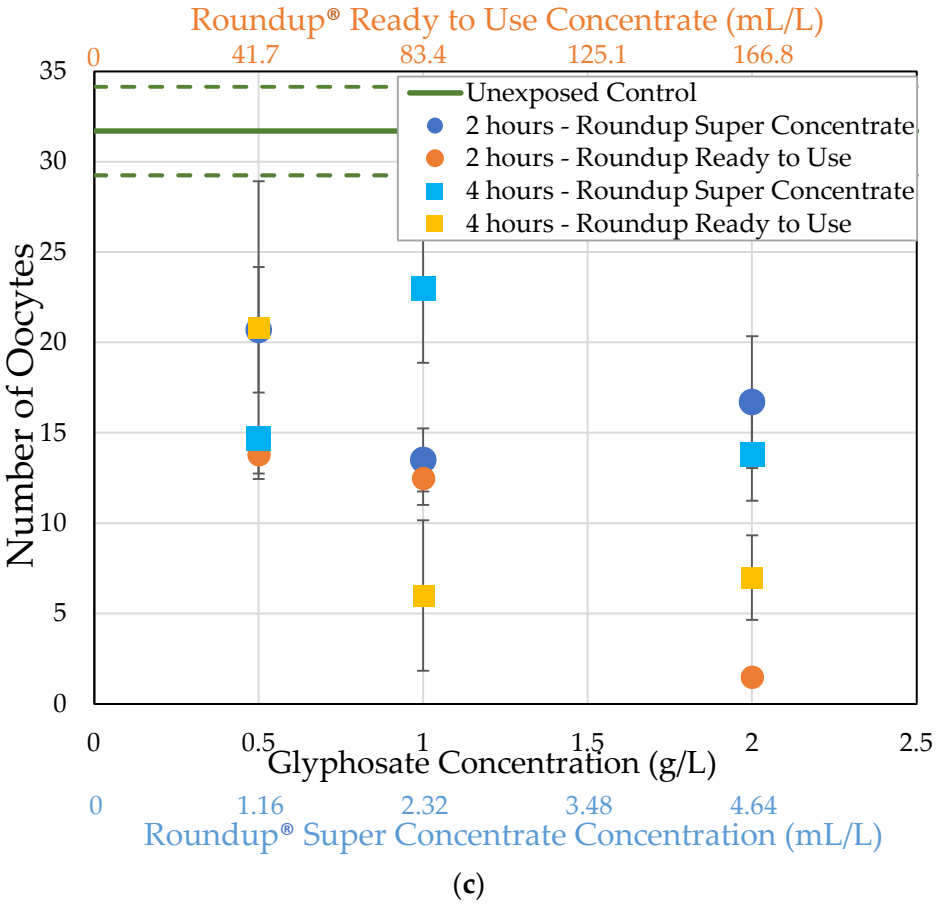
**Figure S1.** Distribution of Dependent Variables. Bars indicate the distribution of each dependent variable: (**a**) survival, (**b**) body weight, (**c**) ovary volume, (**d**) number of oocytes. In each case, the left panel shows the distribution of all exposure treatments combined with controls indicated by diagonal lines and all others as solid, and the right panel shows the distribution of controls only with the normal distribution fitted only to those data. Lines indicate the normal distribution, and box plots of the same data are shown to the right of each histogram.


Roundup® Ready to Use Concentration (mL/L)
0
41.7
83.4
125.1
166.8
Body Weight (mg)
1.5
1.2
0.9
0.6
0.3
0
Unexposed Control
2 hours - Roundup Super Concentrate
2 hours - Roundup Ready to Use
4 hours - Roundup Super Concentrate
4 hours - Roundup Ready to Use
0
0.5
1
1.5
2
2.5
Glyphosate Concentration (g/L)
0
1.16
2.32
3.48
4.64
Roundup® Super Concentrate Concentration (mL/L)

(**a**)


Roundup® Ready to Use Concentration (mL/L)
0
41.7
83.4
125.1
166.8
Ovary Volume (nL)
200
160
120
80
40
0
Unexposed Control
2 hours - Roundup Super Concentrate
2 hours - Roundup Ready to Use
4 hours - Roundup Super Concentrate
4 hours - Roundup Ready to Use
0
0.5
1
1.5
2
2.5
Glyphosate Concentration (g/L)
0
1.16
2.32
3.48
4.64
Roundup® Super Concentrate Concentration (mL/L)

(**b**)




(**c**)

**Figure S2.** Comparison of responses to Roundup® exposure starting at 2 vs. 4 hours after eclosion. Average body weight (**a**), ovary volume (**b**), and number of oocytes (**c**) of *D. melanogaster* females exposed to Roundup Super Concentrate (blue) or Roundup® Ready to Use (orange) in their food medium within either 2 hours (circle) or 4 hours (square) of eclosion for 7 days (± std. error).

**Table S2.** ANOVAs for survival of flies exposed to Roundup® starting by 2 hours after eclosion. These ANOVAs test the effects of glyphosate concentration, pelargonic acid concentration, formulation, herbicide concentration, and the interaction between these on survival of flies exposed to herbicide within 2 hours of eclosion. ANOVA uses least square means identity matrix. Model 1 includes data from controls, while models 2 and 3 necessarily exclude these because of the interaction between formulation and concentration. For whole model, F-value based on Wilks' Lambda. Other F-values based on Exact F Test. Significant *p*-values after Bonferoni correction for three models ($p < 0.017$) shown in bold.

| Model (DF) | Independent Variable | F | *p* |
|---|---|---|---|
| 1 (29) | Whole model | 1.60 | 0.212 |
| | Glyphosate Concentration | 0.22 | 0.641 |
| | Pelargonic Acid Concentration | 2.96 | 0.096 |
| | Glyphosate Concentration X Pelargonic Acid Concentration | 0.72 | 0.404 |
| 2 (24) | Whole model | 1.28 | 0.304 |
| | Formulation | 0.66 | 0.423 |
| | Herbicide Concentration | 0.48 | 0.497 |
| | Formulation X Herbicide Concentration | 0.23 | 0.638 |
| 3 (24) | Whole model | 1.28 | 0.304 |
| | Formulation | 3.45 | 0.076 |
| | Glyphosate Concentration | 0.46 | 0.503 |
| | Glyphosate Concentration X Pelargonic Acid Concentration | 0.10 | 0.755 |

**Table S3.** MANOVAs for all Roundup® exposure and age treatments combined. Results of MANOVAs testing the effects of exposure age, formulation, total herbicide concentration, glyphosate concentration, pelargonic acid concentration and the interaction between these on body weight, ovary volume and number of mature oocytes. MANOVA uses least square means identity matrix. Model 1 includes data from controls, while models 2 and 3 necessarily exclude these because of the interaction between formulation and concentration. For whole model, F-value based on Wilks' Lambda. Other F-values based on Exact F Test. Significant *p*-values after Bonferoni correction for three models ($p < 0.017$) shown in bold.

| Response Variable | Model (DF) | Independent Variable | F | *p* |
|---|---|---|---|---|
| Overall | 1 (139) | Whole Model | 4.86 | **<0.0001** |
| | | Exposure Age | 2.02 | 0.113 |
| | | Formulation | 4.08 | **0.0083** |
| | | Herbicide Concentration | 4.94 | **0.0027** |
| | | Formulation X Herbicide Concentration | 1.61 | 0.189 |
| | 2 (139) | Whole Model | 4.86 | **<0.0001** |
| | | Exposure Age | 2.02 | 0.113 |
| | | Formulation | 7.37 | **0.0001** |
| | | Glyphosate Concentration | 6.92 | **0.0002** |
| | | Formulation X Glyphosate Concentration | 2.78 | 0.0433 |
| | 3 (157) | Whole Model | 6.83 | **<0.0001** |
| | | Exposure Age | 0.823 | 0.483 |
| | | Glyphosate Concentration | 4.84 | **0.003** |
| | | Pelargonic Acid Concentration | 6.65 | **0.0003** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 1.37 | 0.254 |
| Body Weight | 1 (139) | Whole Model | 3.78 | **0.006** |
| | | Exposure Age | 0.0055 | 0.941 |
| | | Formulation | 0.0094 | 0.923 |
| | | Herbicide Concentration | 5.49 | 0.0205 |
| | | Formulation X Herbicide Concentration | 0.25 | 0.618 |
| | 2 (139) | Whole Model | 3.78 | **0.006** |
| | | Exposure Age | 0.0055 | 0.941 |
| | | Formulation | 3.21 | 0.075 |
| | | Glyphosate Concentration | 8.82 | **0.0035** |
| | | Formulation X Glyphosate Concentration | 2.3 | 0.132 |
| | 3 (157) | Whole Model | 5.9 | **0.0002** |
| | | Exposure Age | 0.0125 | 0.911 |
| | | Glyphosate Concentration | 5.82 | **0.017** |
| | | Pelargonic Acid Concentration | 1.26 | 0.264 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.104 | 0.747 |
| Ovary Volume | 1 (139) | Whole Model | 9.36 | **<0.0001** |
| | | Exposure Age | 4.06 | 0.046 |
| | | Formulation | 7.21 | **0.0081** |
| | | Herbicide Concentration | 0.878 | 0.35 |
| | | Formulation X Herbicide Concentration | 4.04 | 0.0463 |
| | 2 (139) | Whole Model | 9.36 | **<0.0001** |
| | | Exposure Age | 4.06 | 0.0457 |
| | | Formulation | 18.9 | **<0.0001** |
| | | Glyphosate Concentration | 3.62 | 0.0593 |
| | | Formulation X Glyphosate Concentration | 7.56 | **0.0068** |
| | 3 (157) | Whole Model | 12.1 | **<0.0001** |
| | | Exposure Age | 1.31 | 0.254 |

| | | | | |
|---|---|---|---|---|
| | | Glyphosate Concentration | 1.25 | 0.266 |
| | | Pelargonic Acid Concentration | 14.4 | **0.0002** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 1.49 | 0.224 |
| Number of Oocytes | 1 (139) | Whole Model | 11.51 | **<0.0001** |
| | | Exposure Age | 1.87 | 0.1738 |
| | | Formulation | 2.01 | 0.159 |
| | | Herbicide Concentration | 8.14 | **0.005** |
| | | Formulation X Herbicide Concentration | 1.78 | 0.185 |
| | 2 (139) | Whole Model | 11.5 | **<0.0001** |
| | | Exposure Age | 1.87 | 0.174 |
| | | Formulation | 17.8 | **<0.0001** |
| | | Glyphosate Concentration | 15.2 | **0.0001** |
| | | Formulation X Glyphosate Concentration | 7.31 | **0.0077** |
| | 3 (157) | Whole Model | 19.8 | **<0.0001** |
| | | Exposure Age | 0.342 | 0.559 |
| | | Glyphosate Concentration | 8.22 | **0.0047** |
| | | Pelargonic Acid Concentration | 15.7 | **0.0001** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 1.83 | 0.178 |

**Table S4.** Separate MANOVAs for exposure to Roundup® starting at 4 hours or 2 hours after eclosion. Results of MANOVAs testing the effects of formulation, total herbicide concentration, glyphosate concentration, pelargonic acid concentration, and the interaction between these on body weight, ovary volume, and number of oocytes of flies exposed to herbicide within 4 h of eclosion (a) or 2 h of eclosion (b). Least squares, mean, identity matrix. Model 1 includes data from controls, while models 2 and 3 necessarily exclude these because of the interaction between formulation and concentration. For whole models, F-ratio based on Wilks' Lambda. Other F-values based on Exact F Test. Significant *p*-values after Bonferoni correction for three models ($p < 0.017$) shown in bold.

**(a) Flies Exposed within 4 h of Eclosion**

| Response Variable | Model (DF) | Independent Variable | F | *p* |
|---|---|---|---|---|
| Overall | 1 (34) | Whole Model | 1.85 | 0.0706 |
| | | Formulation | 3.17 | 0.0367 |
| | | Herbicide Concentration | 1.64 | 0.199 |
| | | Formulation X Herbicide Concentration | 0.873 | 0.465 |
| | 2 (34) | Whole Model | 1.85 | 0.071 |
| | | Formulation | 3.08 | 0.0404 |
| | | Glyphosate Concentration | 1.79 | 0.168 |
| | | Formulation X Glyphosate Concentration | 0.87 | 0.468 |
| | 3 (34) | Whole Model | 1.9 | 0.063 |
| | | Glyphosate Concentration | 2.38 | 0.0871 |
| | | Pelargonic Acid Concentration | 2.03 | 0.128 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.337 | 0.798 |
| Body Weight | 1 (36) | Whole Model | 2.02 | 0.129 |
| | | Formulation | 5.24 | 0.028 |
| | | Herbicide Concentration | 3.85 | 0.576 |
| | | Formulation X Herbicide Concentration | 0.328 | 0.57 |
| | 2 (36) | Whole Model | 2.02 | 0.129 |
| | | Formulation | 2.08 | 0.158 |
| | | Glyphosate Concentration | 4.24 | 0.0467 |
| | | Formulation X Glyphosate Concentration | 0.0089 | 0.926 |
| | 3 | Whole Model | 2.05 | 0.124 |

| | | | | |
|---|---|---|---|---|
| | (36) | Glyphosate Concentration | 6.02 | 0.0191 |
| | | Pelargonic Acid Concentration | 1.82 | 0.185 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.641 | 0.429 |
| Ovary Volume | 1 (36) | Whole Model | 1.9 | 0.148 |
| | | Formulation | 0.4 | 0.532 |
| | | Herbicide Concentration | 0.518 | 0.476 |
| | | Formulation X Herbicide Concentration | 1 | 0.324 |
| | 2 (36) | Whole Model | 1.9 | 0.148 |
| | | Formulation | 2.09 | 0.157 |
| | | Glyphosate Concentration | 1.5 | 0.229 |
| | | Formulation X Glyphosate Concentration | 2.08 | 0.158 |
| | 3 (36) | Whole Model | 1.18 | 0.169 |
| | | Glyphosate Concentration | 0.111 | 0.741 |
| | | Pelargonic Acid Concentration | 0.75 | 0.392 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.065 | 0.8 |
| Number of Oocytes | 1 (36) | Whole Model | 2.04 | 0.125 |
| | | Formulation | 0.0002 | 0.988 |
| | | Herbicide Concentration | 2.45 | 0.126 |
| | | Formulation X Herbicide Concentration | 0.078 | 0.782 |
| | 2 (36) | Whole Model | 2.04 | 0.125 |
| | | Formulation | 1.49 | 0.23 |
| | | Glyphosate Concentration | 3.72 | 0.0616 |
| | | Formulation X Glyphosate Concentration | 0.868 | 0.358 |
| | 3 (36) | Whole Model | 2.03 | 0.127 |
| | | Glyphosate Concentration | 0.849 | 0.363 |
| | | Pelargonic Acid Concentration | 0.971 | 0.331 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.0306 | 0.862 |

**(b) Flies Exposed within 2 h of Eclosion**

| **Response Variable** | **Model (DF)** | **Independent Variable** | **F** | ***p*** |
|---|---|---|---|---|
| Overall | 1 (98) | Whole Model | 5.47 | **<0.0001** |
| | | Formulation | 3.94 | **0.0106** |
| | | Herbicide Concentration | 5.31 | **0.002** |
| | | Formulation X Herbicide Concentration | 1.05 | 0.373 |
| | 2 (98) | Whole Model | 5.47 | **<0.0001** |
| | | Formulation | 6.67 | **0.0004** |
| | | Glyphosate Concentration | 7.7 | **0.0001** |
| | | Formulation X Glyphosate Concentration | 2.32 | 0.0802 |
| | 3 (116) | Whole Model | 8.81 | **<0.0001** |
| | | Glyphosate Concentration | 5 | **0.0027** |
| | | Pelargonic Acid Concentration | 5.15 | **0.0022** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 1.42 | 0.242 |
| Body Weight | 1 (100) | Whole Model | 6.61 | **0.0004** |
| | | Formulation | 2.23 | 0.139 |
| | | Herbicide Concentration | 2.18 | 0.149 |
| | | Formulation X Herbicide Concentration | 1.15 | 0.287 |
| | 2 (100) | Whole Model | 6.61 | **0.0004** |
| | | Formulation | 10.5 | **0.0016** |
| | | Glyphosate Concentration | 4.67 | 0.0331 |

| | | | | |
|---|---|---|---|---|
| | | Formulation X Glyphosate Concentration | 3.45 | 0.0664 |
| | 3 (118) | Whole Model | 9.32 | **<0.0001** |
| | | Glyphosate Concentration | 1.82 | 0.18 |
| | | Pelargonic Acid Concentration | 4.45 | 0.037 |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 0.313 | 0.86 |
| Ovary Volume | 1 (100) | Whole Model | 9.43 | **<0.0001** |
| | | Formulation | 8.56 | **0.0042** |
| | | Herbicide Concentration | 0.345 | 0.558 |
| | | Formulation X Herbicide Concentration | 3.14 | 0.0795 |
| | 2 (100) | Whole Model | 9.43 | **<0.0001** |
| | | Formulation | 19.7 | **<0.0001** |
| | | Glyphosate Concentration | 1.97 | 0.163 |
| | | Formulation X Glyphosate Concentration | 5.5 | 0.021 |
| | 3 (118) | Whole Model | 15.1 | **<0.0001** |
| | | Glyphosate Concentration | 1.26 | 0.264 |
| | | Pelargonic Acid Concentration | 13.9 | **0.0003** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 2.02 | 0.158 |
| Number of Oocytes | 1 (100) | Whole Model | 13.1 | **<0.0001** |
| | | Formulation | 3.04 | 0.0841 |
| | | Herbicide Concentration | 5.3 | 0.0225 |
| | | Formulation X Herbicide Concentration | 2.11 | 0.149 |
| | 2 (100) | Whole Model | 13.1 | **<0.0001** |
| | | Formulation | 18.5 | **<0.0001** |
| | | Glyphosate Concentration | 11.2 | **0.0012** |
| | | Formulation X Glyphosate Concentration | 7.04 | **0.0093** |
| | 3 (118) | Whole Model | 26.1 | **<0.0001** |
| | | Glyphosate Concentration | 7.73 | **0.0063** |
| | | Pelargonic Acid Concentration | 14.6 | **0.0002** |
| | | Glyphosate Concentration X Pelargonic Acid Concentration | 1.48 | 0.226 |

**Table S5.** ANOVAs for body weight of flies exposed to Roundup® starting by 2 hours after eclosion. Results of ANOVAs testing the effects of formulation, total herbicide concentration, glyphosate concentration, pelargonic acid concentration, and the interaction between these on body weight of flies exposed to herbicide within 2 hours of eclosion (DF = 127). ANOVA uses least square means identity matrix. For whole model, F-value based on Wilks' Lambda. Other F-values based on Exact F Test. Significant *p*-values after Bonferoni correction for three models ($p < 0.017$) shown in bold.

| Model | Independent Variable | F | *p* |
|---|---|---|---|
| 1 | Whole Model | 8.03 | **<0.0001** |
| | Formulation | 12.9 | **0.0005** |
| | Herbicide Concentration | 4.56 | 0.0349 |
| | Formulation X Herbicide Concentration | 5.66 | 0.0191 |
| 2 | Whole Model | 6.2 | **0.0005** |
| | Formulation | 0.865 | 0.354 |
| | Glyphosate Concentration | 3.51 | 0.0629 |
| | Formulation X Glyphosate Concentration | 0.933 | 0.336 |
| 3 | Whole Model | 9.13 | **<0.0001** |
| | Glyphosate Concentration | 2.8 | 0.0962 |
| | Pelargonic Acid Concentration | 5.63 | 0.0187 |
| | Glyphosate Concentration X Pelargonic Acid Concentration | 0.874 | 0.768 |

**Table S6.** MANOVAs for reproductive response variables of flies exposed to Roundup® starting by 2 hours after eclosion. Results of MANOVAs testing the effects of formulation, total herbicide concentration, glyphosate concentration, pelargonic acid concentration, and the interaction between these on the reproductive response variables (ovary size and number of oocytes) on flies exposed to herbicide within 2 hours of eclosion. MANOVA uses least square means identity matrix. For whole model, F-value based on Wilks' Lambda. Other F-values based on Exact F Test. Significant *p*-values after Bonferoni correction for two models (overall and by formulation; $p < 0.025$) shown in bold.

| Formulation | Response Variable | *DF* | Independent Variable | F | *p* |
|---|---|---|---|---|---|
| Both Formulations | Overall | 99 | Whole model | 8.21 | **<0.0001** |
| | | | Formulation | 10.1 | **0.0001** |
| | | | Glyphosate Concentration | 11.0 | **<0.0001** |
| | | | Formulation * Glyphosate Concentration | 3.49 | 0.034 |
| | Ovary Volume | 100 | Whole model | 9.43 | **<0.0001** |
| | | | Formulation | 19.7 | **<0.0001** |
| | | | Glyphosate Concentration | 1.97 | **0.163** |
| | | | Formulation * Glyphosate Concentration | 5.50 | **0.021** |
| | Number of Oocytes | 100 | Whole model | 13.1 | **<0.0001** |
| | | | Formulation | 18.5 | **<0.0001** |
| | | | Glyphosate Concentration | 11.2 | **0.0012** |
| | | | Formulation * Glyphosate Concentration | 7.04 | **0.0093** |
| Super Concentrate | Overall | 72 | Glyphosate Concentration | 1.79 | 0.174 |
| | Ovary size | 73 | Glyphosate Concentration | 0.222 | 0.639 |
| | # oocytes | 73 | Glyphosate Concentration | 0.532 | 0.468 |
| Ready to Use | Overall | 66 | Glyphosate Concentration | 18.0 | **<0.0001** |
| | Ovary size | 67 | Glyphosate Concentration | 27.3 | **<0.0001** |
| | # oocytes | 67 | Glyphosate Concentration | 35.8 | **<0.0001** |