

Supplementary information to:

Original article:

NEUROTOXICITY OF MANCOZEB-BASED COMMERCIAL FUNGICIDE IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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ABSTRACT

Mancozeb, a polymeric dithiocarbamate complex fungicide with zinc and manganese salts, has the potential to be neurotoxic to humans. Unfortunately, the parent molecule maneb has attracted far too much attention, limiting the available evidence on mancozeb neurotoxicity to preclinical research and non-human cells. We sought to evaluate mancozeb cytotoxicity in neuroblastoma SH-SY5Y cells at lower concentrations than those used for maneb in *in vitro* investigations in order to quantify its risk for humans. Commercial mancozeb showed concentration- and time-dependent neurotoxicity in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction test (EC_{50} = 5.9 μ M and 1.7 μ M at 24 h and 72 h respectively). Using the trypan blue exclusion dye, cell death toll reached around 100% after 24- and 72-hour exposure to mancozeb 1 μ M and 0.5 μ M respectively. Reactive oxygen species generated by mancozeb, which peaked at 4 μ M, could be the cause of cell death. The number and length of neurites were concentration-dependently reduced by mancozeb at sub- μ M concentrations, and this was accompanied by changes in cell biomechanical characteristics (stiffness) as determined by atomic force microscopy. The uncertainty factor obtained from our cytotoxic studies, when performing risk assessment of mancozeb, varied from 200 to 2000, which may result in detectable neurotoxicity in humans in accordance with international regulatory agencies recommendations.

Keywords: Atomic force microscopy, cytotoxicity tests, mancozeb, neurons, oxidative stress, zinc

ANOVA OF MTT DATA (24 H)

```

> library(readxl)
> MTT_D1 <- read_excel("MTT_D1.xlsx", col_types = c("text",
+                                                    "text", "numeric", "numeric"))
> View(MTT_D1)
> attach(MTT_D1)
> names(MTT_D1)
[1] "Dose"          "Replicate"      "Response"        "Normalized_data"
> class("Dose")
[1] "character"
> factor(Dose)
[1] 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 4 4 4 4
4 4 4
[32] 4 8 8 8 8 8 8 8 8 16 16 16 16 16 16 16 16
Levels: 0 1 16 2 4 8
> summary(MTT_D1)
      Dose          Replicate          Response          Normalized_data
Length:48          Length:48          Min.   :-0.0003333          Min.   : -0.1096
Class :character   Class :character          1st Qu.: 0.0844167          1st Qu.: 27.7686
Mode  :character   Mode  :character          Median : 0.2533333          Median : 83.3333
                                Mean   : 0.2075069          Mean   : 68.2589
                                3rd Qu.: 0.2921667          3rd Qu.: 96.1075
                                Max.   : 0.4083333          Max.   :134.3202

> aov(Normalized_data~Dose)
Call:
aov(formula = Normalized_data ~ Dose)

Terms:
              Dose Residuals
Sum of Squares 57244.38  8921.46
Deg. of Freedom      5      42

Residual standard error: 14.57449
Estimated effects may be unbalanced
> AOV_D1_MTT <- aov(Normalized_data~Dose)
> summary(AOV_D1_MTT)
      Df Sum Sq Mean Sq F value Pr(>F)
Dose      5  57244  11449   53.9 <2e-16 ***
Residuals 42   8921    212
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(AOV_D1_MTT)
Tukey multiple comparisons of means
 95% family-wise confidence level

Fit: aov(formula = Normalized_data ~ Dose)

$Dose
      diff          lwr          upr          p adj
1-0  -5.441338 -27.195576  16.3129002  0.9746853
16-0 -88.884320 -110.638558 -67.1300823  0.0000000
2-0  -6.661184 -28.415422  15.0930537  0.9407204
4-0 -20.805921 -42.560159   0.9483169  0.0680144
8-0 -69.723136 -91.477374 -47.9688980  0.0000000
16-1 -83.442982 -105.197220 -61.6887445  0.0000000
2-1  -1.219846 -22.974084  20.5343914  0.9999803
4-1 -15.364583 -37.118821   6.3896546  0.3027833
8-1 -64.281798 -86.036036 -42.5275603  0.0000000
2-16  82.223136  60.468898  103.9773739  0.0000000
4-16  68.078399  46.324161  89.8326370  0.0000000
8-16  19.161184  -2.593054  40.9154221  0.1124992
4-2 -14.144737 -35.898975   7.6095011  0.3925616
8-2 -63.061952 -84.816190 -41.3077138  0.0000000
8-4 -48.917215 -70.671453 -27.1629770  0.0000005

```

(72 H)

```
> MTT_D2 <- read_excel("MTT_D2.xlsx", col_types = c("text",
+                                                    "text", "numeric", "numeric"))
> View(MTT_D2)
> attach(MTT_D2)
> names(MTT_D2)
[1] "Dose"           "Replicate"      "Response"       "Normalized_data"
> class("Dose")
[1] "character"
> factor(Dose)
[1] 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 4 4
4 4 4 4 4
[32] 4 8 8 8 8 8 8 8 8 8 16 16 16 16 16 16 16 16
Levels: 0 1 16 2 4 8
> summary(MTT_D2)
      Dose           Replicate           Response           Normalized_data
Length:48          Length:48          Min.   :-0.01933          Min.   : 0.00
Class :character    Class :character          1st Qu.: 0.02800          1st Qu.: 10.53
Mode  :character    Mode  :character          Median : 0.07983          Median : 30.01
                                Mean   : 0.11608          Mean   : 43.85
                                3rd Qu.: 0.20350          3rd Qu.: 76.50
                                Max.   : 0.30367          Max.   :114.16

> aov(Normalized_data~Dose)
Call:
aov(formula = Normalized_data ~ Dose)

Terms:
          Dose Residuals
Sum of Squares 60123.02  5037.03
Deg. of Freedom      5      42

Residual standard error: 10.95123
Estimated effects may be unbalanced
> AOV_D2_MTT <- aov(Normalized_data~Dose)
> summary(AOV_D2_MTT)
      Df Sum Sq Mean Sq F value Pr(>F)
Dose      5 60123  12025  100.3 <2e-16 ***
Residuals 42  5037    120
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(AOV_D2_MTT)
Tukey multiple comparisons of means
 95% family-wise confidence level

Fit: aov(formula = Normalized_data ~ Dose)

$Dose
      diff          lwr          upr          p adj
1-0 -20.551378 -36.89745 -4.205308 0.0065765
16-0 -87.562657 -103.90873 -71.216586 0.0000000
2-0 -52.600251 -68.94632 -36.254180 0.0000000
4-0 -82.581454 -98.92752 -66.235383 0.0000000
8-0 -93.530702 -109.87677 -77.184632 0.0000000
16-1 -67.011278 -83.35735 -50.665208 0.0000000
2-1 -32.048872 -48.39494 -15.702802 0.0000092
4-1 -62.030075 -78.37615 -45.684005 0.0000000
8-1 -72.979323 -89.32539 -56.633253 0.0000000
2-16 34.962406  18.61634  51.308476 0.0000016
4-16  4.981203 -11.36487  21.327273 0.9418638
8-16 -5.968045 -22.31412  10.378025 0.8826461
4-2 -29.981203 -46.32727 -13.635133 0.0000318
8-2 -40.930451 -57.27652 -24.584381 0.0000000
8-4 -10.949248 -27.29532  5.396822 0.3597055
```

```

> library(readxl)
> MTT_D5 <- read_excel("MTT_D5.xlsx", col_types = c("text",
+                                                    "text", "numeric", "numeric"))
> View(MTT_D5)
> attach(MTT_D5)
> names(MTT_D5)
[1] "Dose"          "Replicate"      "Response"       "Normalized_data"
> class("Dose")
[1] "character"
> factor(Dose)
[1] 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 4 4
4 4 4 4 4
[32] 4 8 8 8 8 8 8 8 8 16 16 16 16 16 16 16 16
Levels: 0 1 16 2 4 8
> summary(MTT_D5)
      Dose          Replicate          Response          Normalized_data
Length:48      Length:48      Min.   :-0.044667      Min.   :-16.5432
Class :character Class :character  1st Qu.: 0.002417      1st Qu.: 0.8951
Mode  :character Mode  :character  Median : 0.111333      Median : 41.2346
                          Mean   : 0.116021      Mean   : 42.9707
                          3rd Qu.: 0.208000      3rd Qu.: 77.0370
                          Max.   : 0.389667      Max.   :144.3210

> aov(Normalized_data~Dose)
Call:
aov(formula = Normalized_data ~ Dose)

Terms:
              Dose Residuals
Sum of Squares 89197.64 11396.98
Deg. of Freedom    5      42

Residual standard error: 16.4729
Estimated effects may be unbalanced
> AOV_D5_MTT <- aov(Normalized_data~Dose)
> summary(AOV_D5_MTT)
      Dose      Df Sum Sq Mean Sq F value Pr(>F)
Residuals  42 11397    271
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(AOV_D5_MTT)
Tukey multiple comparisons of means
 95% family-wise confidence level

Fit: aov(formula = Normalized_data ~ Dose)

$Dose
      diff      lwr      upr      p adj
1-0    -7.1450617 -31.73292  17.442798 0.9521490
16-0  -102.2993827 -126.88724 -77.711523 0.0000000
2-0   -40.9567901 -65.54465 -16.368931 0.0001610
4-0   -89.8456790 -114.43354 -65.257820 0.0000000
8-0  -102.0216049 -126.60946 -77.433746 0.0000000
16-1  -95.1543210 -119.74218 -70.566462 0.0000000
2-1   -33.8117284 -58.39959  -9.223869 0.0023622
4-1   -82.7006173 -107.28848 -58.112758 0.0000000
8-1   -94.8765432 -119.46440 -70.288684 0.0000000
2-16   61.3425926   36.75473  85.930452 0.0000000
4-16   12.4537037  -12.13416  37.041563 0.6586421
8-16    0.2777778  -24.31008  24.865637 1.0000000
4-2   -48.8888889  -73.47675 -24.301030 0.0000070
8-2   -61.0648148  -85.65267 -36.476955 0.0000001
8-4   -12.1759259  -36.76379  12.411933 0.6795473

```

MTT statistics 24 and 48 h

```
> tidy(LL.4Model_MTT_Total)
# A tibble: 8 × 6
  term      curve estimate std.error statistic  p.value
<chr>    <chr>    <dbl>    <dbl>    <dbl>    <dbl>
1 Hill Slope A      3.55     0.636     5.58 0.00506
2 Hill Slope B      2.47     0.368     6.72 0.00255
3 Min      A      8.76     4.83     1.81 0.144
4 Min      B      8.14     2.80     2.91 0.0438
5 Max      A     97.0     2.30    42.1 0.00000191
6 Max      B     99.4     3.61    27.5 0.0000103
7 EC50     A      5.86     0.396    14.8 0.000121
8 EC50     B      1.73     0.129    13.5 0.000175
```

```
> compParm(LL.4Model_MTT_Total, "EC50", "-")
```

Comparison of parameter 'EC50'

	Estimate	Std. Error	t-value	p-value
A-B	4.12702	0.41634	9.9125	0.0005814 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANALYSIS OF VARIANCE OF TRYPAN BLUE DATA

```
> library(readxl)
> Trypan_Blue_1_3_and_5_days_ <- read_excel("C:/Users/EVELIN CUADRO/Desktop/Datos MCZ Toxicity/Trypan Blue (1 3 and 5 days).xlsx",
+     col_types = c("text", "numeric", "numeric",
+                   "numeric"))
> View(Trypan_Blue_1_3_and_5_days_)
> attach(Trypan_Blue_1_3_and_5_days_)
> names(Trypan_Blue_1_3_and_5_days_)
[1] "Concentration"      "D1PercentageDeathCells" "D3PercentageDeathCells"
[4] "D5PercentageDeathCells"
> factor(Concentration)
 [1] 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.1µM 0.1µM
[14] 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM
[27] 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM
[40] 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM
[53] 1µM 1µM 1µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM
[66] 2µM
Levels: 0.0µM 0.1µM 0.2µM 0.5µM 1µM 2µM
> summary(Trypan_Blue_1_3_and_5_days_)
  Concentration      D1PercentageDeathCells D3PercentageDeathCells D5PercentageDeathCells
Length:66          Min.      : 0.00          Min.      : 1.01          Min.      : 0.00
Class :character   1st Qu.: 12.75          1st Qu.: 14.71          1st Qu.: 14.67
Mode  :character   Median : 40.68          Median : 71.63          Median : 69.77
                          Mean      : 50.80          Mean      : 58.81          Mean      : 58.04
                          3rd Qu.:100.00          3rd Qu.:100.00          3rd Qu.:100.00
                          Max.      :100.00          Max.      :100.00          Max.      :100.00

> aov(D1PercentageDeathCells~Concentration)
Call:
aov(formula = D1PercentageDeathCells ~ Concentration)

Terms:
              Concentration Residuals
Sum of Squares      98121.37  10044.83
Deg. of Freedom           5      60

Residual standard error: 12.93885
Estimated effects may be unbalanced

> aov(D3PercentageDeathCells~Concentration)
Call:
aov(formula = D3PercentageDeathCells ~ Concentration)

Terms:
              Concentration Residuals
Sum of Squares     106975.48   3594.88
Deg. of Freedom           5      60

Residual standard error: 7.740452
Estimated effects may be unbalanced

> aov(D5PercentageDeathCells~Concentration)
Call:
aov(formula = D5PercentageDeathCells ~ Concentration)

Terms:
              Concentration Residuals
Sum of Squares     114958.39   1745.15
Deg. of Freedom           5      60

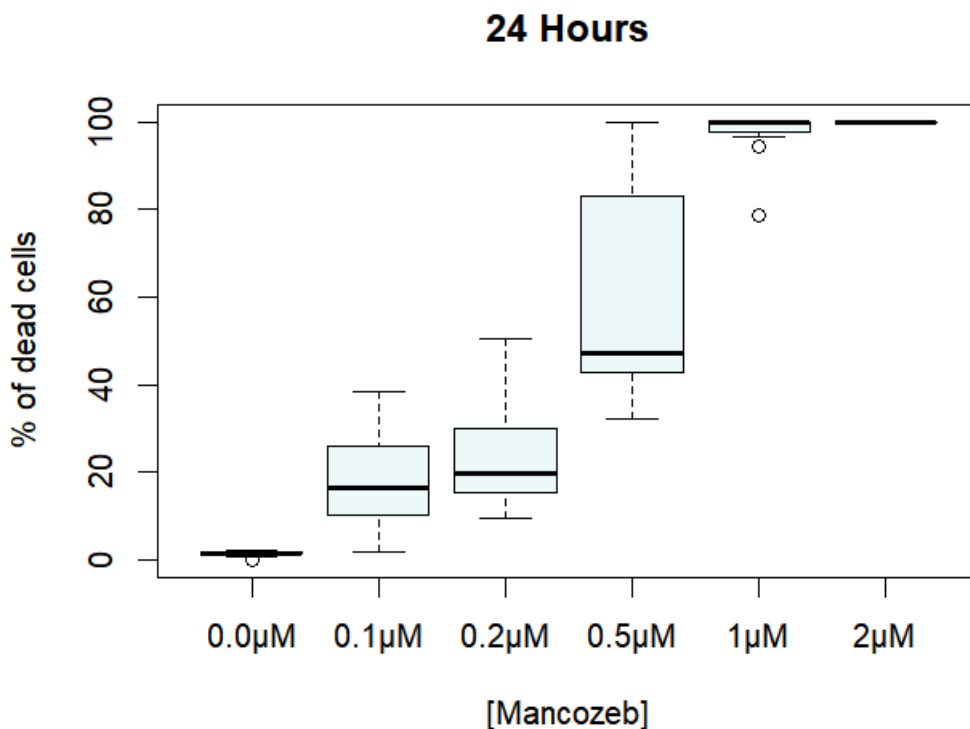
Residual standard error: 5.393131
Estimated effects may be unbalanced
> AOV_TB_D1 <- aov(D1PercentageDeathCells~Concentration)
> AOV_TB_D3 <- aov(D3PercentageDeathCells~Concentration)
```

```

> AOV_TB_D5 <- aov(D5PercentageDeathCells~Concentration)
> summary(AOV_TB_D1)
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5  98121   19624   117.2 <2e-16 ***
Residuals    60  10045     167
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(AOV_TB_D3)
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5 106975   21395   357.1 <2e-16 ***
Residuals    60   3595     60
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(AOV_TB_D5)
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5 114958   22992   790.5 <2e-16 ***
Residuals    60   1745     29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> boxplot(D1PercentageDeathCells~Concentration,
+         col = rgb(0.82, 0.93, 0.93, alpha = 0.4),
+         xlab = "[Mancozeb]",
+         ylab = "% of dead cells",
+         main = "24 Hours")

```



```

> boxplot(D3PercentageDeathCells~Concentration,
+         col = rgb(0.82, 0.93, 0.93, alpha = 0.4),

```


ANALYSIS OF VARIANCE: NUMBER OF NEURITES PER CELL.

Entre dosis

```
> library(readxl)
> N0Neurites <- read_excel("C:/Users/EVELIN CUADRO/Desktop/Datos MCZ To
xicity/N0Neurites.xlsx",
+   col_types = c("text", "numeric", "numeric",
+   "numeric"))
> view(N0Neurites)
> attach(N0Neurites)

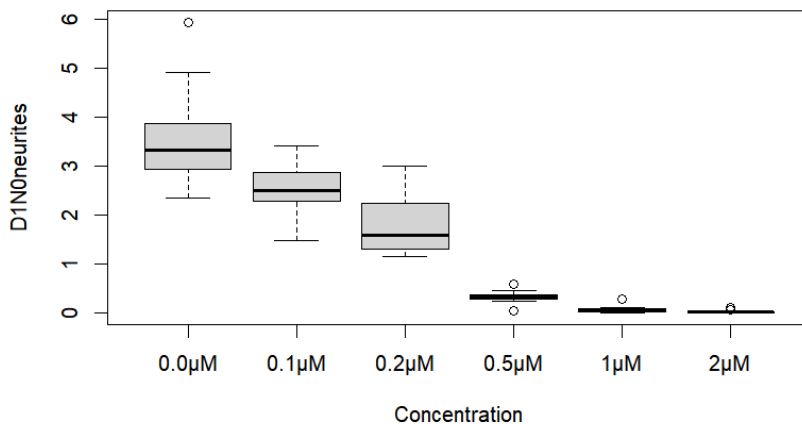
> names(N0Neurites)
[1] "concentration" "D1N0neurites" "D3N0neurites" "D5N0neurites"

> class(Concentration)
[1] "character"
> class(D1N0neurites)
[1] "numeric"
> class(D3N0neurites)
[1] "numeric"
> class(D5N0neurites)
[1] "numeric"

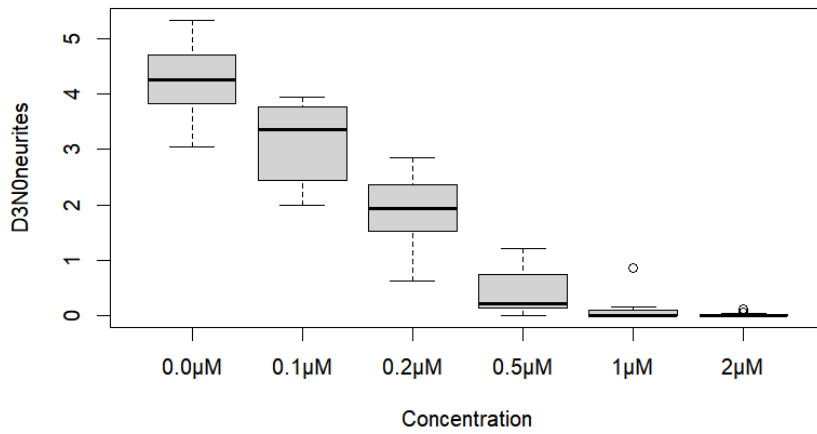
> factor(Concentration)
 [1] 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM
[12] 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM
[23] 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM
[34] 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM
[45] 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM
[56] 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM
Levels: 0.0µM 0.1µM 0.2µM 0.5µM 1µM 2µM

> summary(N0Neurites)
Concentration      D1N0neurites      D3N0neurites      D5N0neurites
Length:66          Min. :0.00000    Min. :0.00000    Min. :0.00000
Class :character   1st Qu.:0.06773  1st Qu.:0.05474  1st Qu.:0.08546
Mode :character    Median :0.86859  Median :1.09704  Median :1.29167
                   Mean :1.38853    Mean :1.65379    Mean :1.35229
                   3rd Qu.:2.47177 3rd Qu.:3.04846 3rd Qu.:2.44949
                   Max. :5.93750    Max. :5.33333    Max. :4.37037

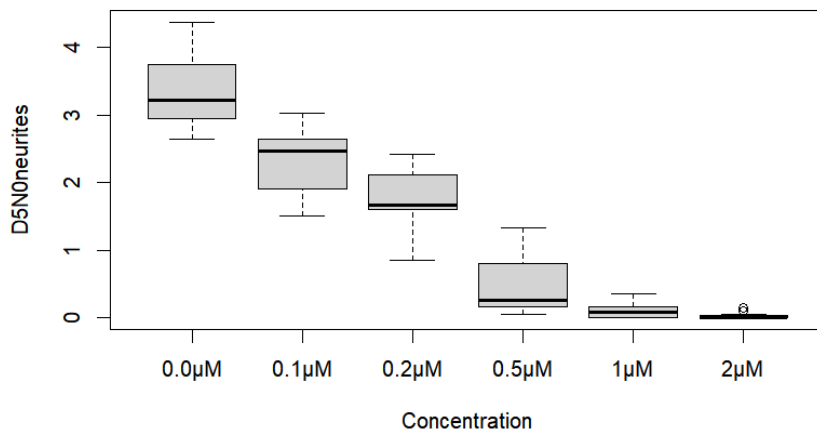
> boxplot(D1N0neurites~Concentration)
```



```
> boxplot(D3N0neurites~Concentration)
```



```
> boxplot(D5N0neurites~Concentration)
```



```
> aov(D1N0neurites~Concentration)
```

```
Call:
aov(formula = D1N0neurites ~ Concentration)
```

```
Terms:
          Concentration Residuals
Sum of Squares    121.26018  17.76454
Deg. of Freedom         5      60
```

```
Residual standard error: 0.5441284
Estimated effects may be unbalanced
```

```
> aov(D3N0neurites~Concentration)
```

```
Call:
aov(formula = D3N0neurites ~ Concentration)
```

```
Terms:
          Concentration Residuals
Sum of Squares    172.04333  17.29542
Deg. of Freedom         5      60
```

```
Residual standard error: 0.5368957
Estimated effects may be unbalanced
```

```
> aov(D5N0neurites~Concentration)
```

```
Call:
aov(formula = D5N0neurites ~ Concentration)
```

```
Terms:
          Concentration Residuals
Sum of Squares    101.46431   9.86062
Deg. of Freedom         5      60
```

```
Residual standard error: 0.4053933
Estimated effects may be unbalanced
```

```
> AOV_D1_NN <- aov(D1N0neurites~Concentration) #Anova del día 1
> AOV_D3_NN <- aov(D3N0neurites~Concentration) #Anova del día 3
> AOV_D5_NN <- aov(D5N0neurites~Concentration) #Anova del día 5
```

```
> summary(AOV_D1_NN)
```

```
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5 121.26  24.252   81.91 <2e-16 ***
Residuals    60  17.76   0.296
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> summary(AOV_D3_NN)
```

```
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5 172.0  34.41  119.4 <2e-16 ***
Residuals    60  17.3   0.29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> summary(AOV_D5_NN)
```

```
      Df Sum Sq Mean Sq F value Pr(>F)
Concentration  5 101.46  20.293  123.5 <2e-16 ***
Residuals    60   9.86   0.164
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> TukeyHSD(AOV_D1_NN)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = D1N0neurites ~ Concentration)
```

```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-1.06730724	-1.7503191	-0.38429542	0.0003111
0.2µM-0.0µM	-1.82555253	-2.5085644	-1.14254071	0.0000000
0.5µM-0.0µM	-3.26778151	-3.9507933	-2.58476969	0.0000000
1µM-0.0µM	-3.52290188	-4.2059137	-2.83989006	0.0000000
2µM-0.0µM	-3.56772068	-4.2507325	-2.88470886	0.0000000
0.2µM-0.1µM	-0.75824529	-1.4412571	-0.07523347	0.0211499
0.5µM-0.1µM	-2.20047426	-2.8834861	-1.51746245	0.0000000
1µM-0.1µM	-2.45559464	-3.1386065	-1.77258282	0.0000000
2µM-0.1µM	-2.50041343	-3.1834253	-1.81740161	0.0000000
0.5µM-0.2µM	-1.44222897	-2.1252408	-0.75921715	0.0000008
1µM-0.2µM	-1.69734935	-2.3803612	-1.01433753	0.0000000
2µM-0.2µM	-1.74216814	-2.4251800	-1.05915632	0.0000000
1µM-0.5µM	-0.25512038	-0.9381322	0.42789144	0.8796328
2µM-0.5µM	-0.29993917	-0.9829510	0.38307265	0.7877997
2µM-1µM	-0.04481879	-0.7278306	0.63819303	0.9999609

```
> TukeyHSD(AOV_D3_NN)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = D3N0neurites ~ Concentration)
```

```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-1.15406132	-1.8279943	-0.4801283	0.0000648
0.2µM-0.0µM	-2.39648696	-3.0704200	-1.7225539	0.0000000
0.5µM-0.0µM	-3.82550314	-4.4994362	-3.1515701	0.0000000
1µM-0.0µM	-4.17127239	-4.8452054	-3.4973394	0.0000000
2µM-0.0µM	-4.26888657	-4.9428196	-3.5949535	0.0000000
0.2µM-0.1µM	-1.24242564	-1.9163587	-0.5684926	0.0000157
0.5µM-0.1µM	-2.67144182	-3.3453749	-1.9975088	0.0000000
1µM-0.1µM	-3.01721107	-3.6911441	-2.3432780	0.0000000
2µM-0.1µM	-3.11482526	-3.7887583	-2.4408922	0.0000000
0.5µM-0.2µM	-1.42901618	-2.1029492	-0.7550831	0.0000007
1µM-0.2µM	-1.77478542	-2.4487185	-1.1008524	0.0000000
2µM-0.2µM	-1.87239961	-2.5463326	-1.1984666	0.0000000
1µM-0.5µM	-0.34576925	-1.0197023	0.3281638	0.6590596
2µM-0.5µM	-0.44338344	-1.1173165	0.2305496	0.3905647
2µM-1µM	-0.09761419	-0.7715472	0.5763188	0.9981178

```
> TukeyHSD(AOV_D5_NN)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = D5N0neurites ~ Concentration)
```

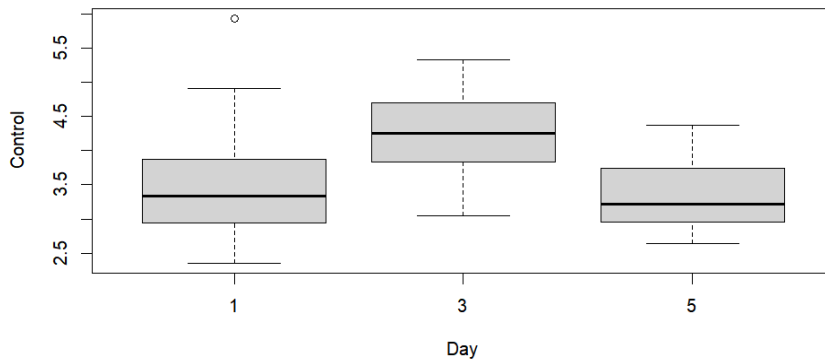
```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-1.05100160	-1.5598675	-0.54213566	0.0000013
0.2µM-0.0µM	-1.60865556	-2.1175215	-1.09978962	0.0000000
0.5µM-0.0µM	-2.86479523	-3.3736612	-2.35592929	0.0000000
1µM-0.0µM	-3.27201700	-3.7808829	-2.76315107	0.0000000
2µM-0.0µM	-3.34450526	-3.8533712	-2.83563932	0.0000000
0.2µM-0.1µM	-0.55765396	-1.0665199	-0.04878803	0.0237512
0.5µM-0.1µM	-1.81379363	-2.3226596	-1.30492770	0.0000000
1µM-0.1µM	-2.22101540	-2.7298813	-1.71214947	0.0000000
2µM-0.1µM	-2.29350366	-2.8023696	-1.78463773	0.0000000
0.5µM-0.2µM	-1.25613967	-1.7650056	-0.74727373	0.0000000
1µM-0.2µM	-1.66336144	-2.1722274	-1.15449551	0.0000000
2µM-0.2µM	-1.73584970	-2.2447156	-1.22698376	0.0000000
1µM-0.5µM	-0.40722177	-0.9160877	0.10164417	0.1886670
2µM-0.5µM	-0.47971003	-0.9885760	0.02915591	0.0756219
2µM-1µM	-0.07248826	-0.5813542	0.43637768	0.9982620

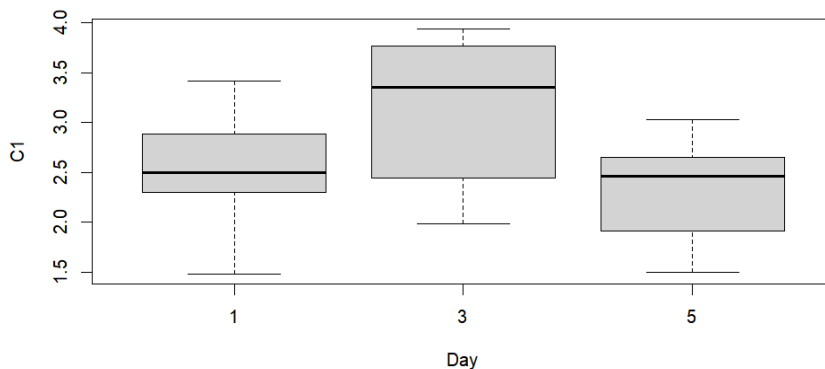
Entre días

```
> library(readxl)
> N0Neurites_by_day_ <- read_excel("C:/Users/EVELIN CUADRO/Desktop/Dato
s MCZ Toxicity/N0Neurites (by day).xlsx",
+   col_types = c("text", "numeric", "numeric",
+                 "numeric", "numeric", "numeric",
+                 "numeric"))
> View(N0Neurites_by_day_)
> attach(N0Neurites_by_day_)
> names(N0Neurites_by_day_)
[1] "Day"      "Control"  "C1"      "C2"      "C3"      "C4"      "C5"
> class(Day)
[1] "character"
> class(Control)
[1] "numeric"
> class(C1)
[1] "numeric"
> class(C2)
[1] "numeric"
> class(C3)
[1] "numeric"
> class(C4)
[1] "numeric"
> class(C5)
[1] "numeric"
> factor(Day)
[1] 1 1 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3 3 3 5 5 5 5 5 5 5 5 5 5
Levels: 1 3 5
```

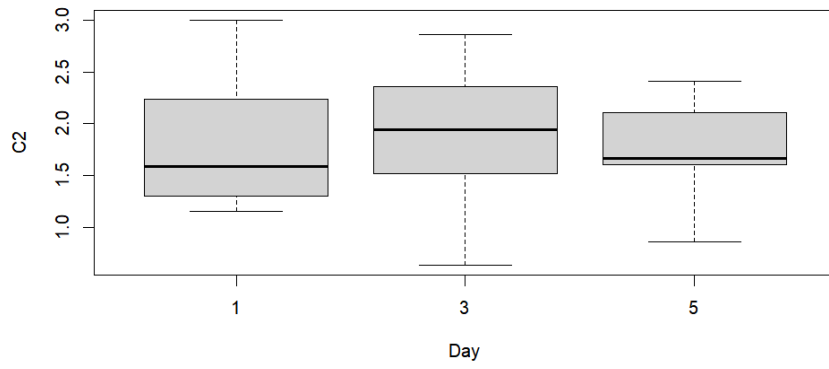
```
> boxplot(Control~Day)
```



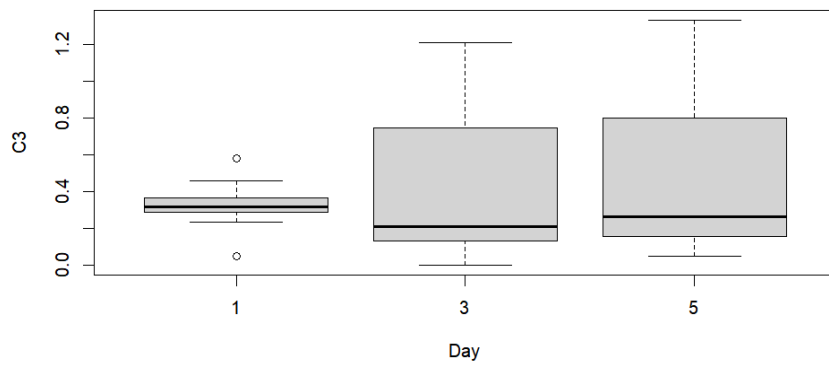
```
> boxplot(C1~Day)
```



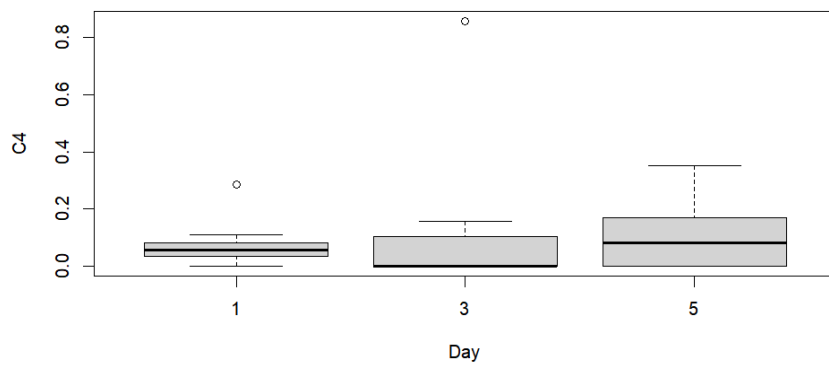
```
> boxplot(C2~Day)
```



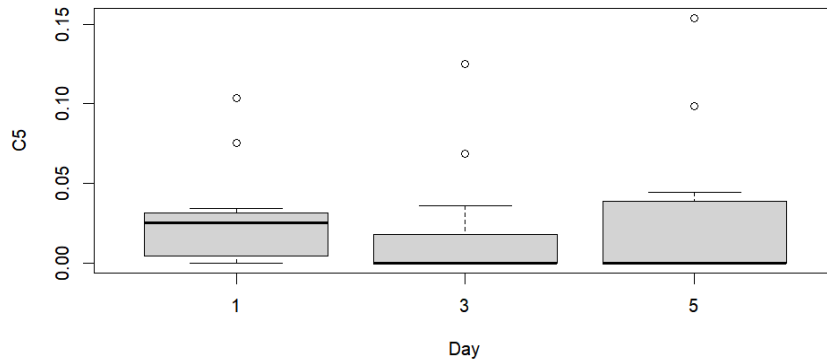
```
> boxplot(C3~Day)
```



```
> boxplot(C4~Day)
```



```
> boxplot(C5~Day)
```



```
> aov(Control~Day)
```

```
Call:
aov(formula = Control ~ Day)
```

```
Terms:
              Day Residuals
Sum of Squares  5.002561 19.278700
Deg. of Freedom    2         30
```

```
Residual standard error: 0.8016379
Estimated effects may be unbalanced
```

```
> aov(C1~Day)
```

```
Call:
aov(formula = C1 ~ Day)
```

```
Terms:
              Day Residuals
Sum of Squares  3.912089 10.094430
Deg. of Freedom    2         30
```

```
Residual standard error: 0.5800698
Estimated effects may be unbalanced
```

```
> aov(C2~Day)
```

```
Call:
aov(formula = C2 ~ Day)
```

```
Terms:
              Day Residuals
Sum of Squares  0.112882 10.218817
Deg. of Freedom    2         30
```

```
Residual standard error: 0.5836328
Estimated effects may be unbalanced
```

```
> aov(C3~Day)
```

```
Call:
aov(formula = C3 ~ Day)
```

```
Terms:
              Day Residuals
Sum of Squares  0.195887  4.424007
Deg. of Freedom    2         30
```

```
Residual standard error: 0.3840142
Estimated effects may be unbalanced
```

```

> aov(C4~Day)
Call:
  aov(formula = C4 ~ Day)

Terms:
              Day Residuals
Sum of Squares 0.0112338 0.8511000
Deg. of Freedom    2         30

Residual standard error: 0.168434
Estimated effects may be unbalanced

> aov(C5~Day)
Call:
  aov(formula = C5 ~ Day)

Terms:
              Day Residuals
Sum of Squares 0.00066568 0.05352666
Deg. of Freedom    2         30

Residual standard error: 0.04224005
Estimated effects may be unbalanced

|> AOV_CONTROL_NN <- aov(Control~Day)
> AOV_C1_NN <- aov(C1~Day)
> AOV_C2_NN <- aov(C2~Day)
> AOV_C3_NN <- aov(C3~Day)
> AOV_C4_NN <- aov(C4~Day)
> AOV_C5_NN <- aov(C5~Day)

> summary(AOV_CONTROL_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  5.003   2.5013   3.892 0.0314 *
Residuals 30 19.279   0.6426
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> summary(AOV_C1_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  3.912   1.9560   5.813 0.00735 **
Residuals 30 10.094   0.3365
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> summary(AOV_C2_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  0.113   0.0564   0.166 0.848
Residuals 30 10.219   0.3406

> summary(AOV_C3_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  0.196   0.09794  0.664 0.522
Residuals 30  4.424   0.14747

> summary(AOV_C4_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  0.0112  0.005617  0.198 0.821
Residuals 30  0.8511  0.028370

> summary(AOV_C5_NN)
      Df Sum Sq Mean Sq F value Pr(>F)
Day      2  0.00067  0.0003328  0.187 0.831
Residuals 30  0.05353  0.0017842

```

Comentado [EGCB1]: Resumen de ANOVAs.
 Comparación entre los datos recolectado a los 1, 3 y 5 días post tratamiento en cada concentración

ANALYSIS OF VARIANCE OF NEURITE LENGTH PER CELL

Entre dosis

```
> library(readxl)
> NeuritesLength_by_dose_ <- read_excel("C:/Users/EVELIN CUADRO/Desktop/Datos MCZ Toxicity/NeuritesLength (by dose).xlsx",
+   col_types = c("text", "numeric", "numeric",
+   "numeric"))

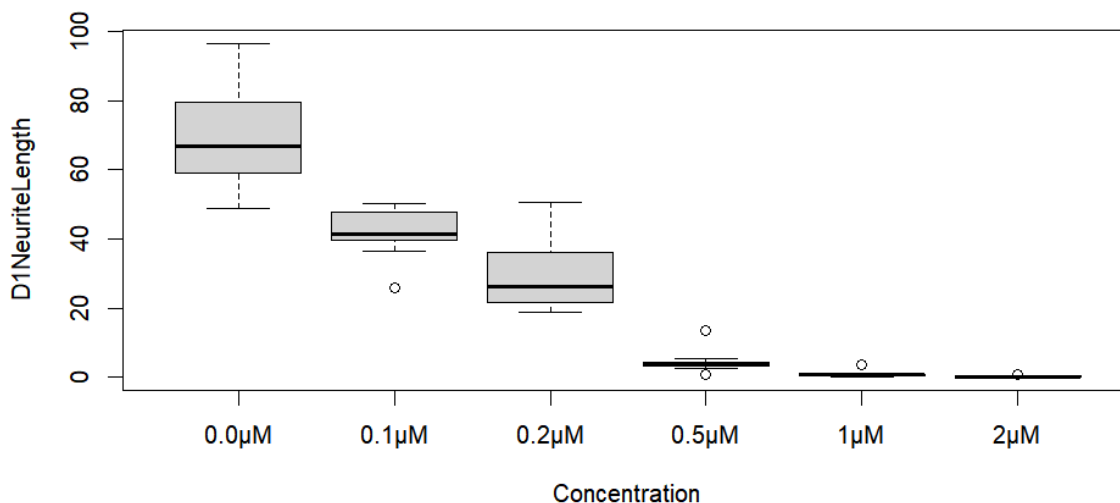
> view(NeuritesLength_by_dose_)
> attach(NeuritesLength_by_dose_)

> names(NeuritesLength_by_dose_)
[1] "Concentration" "D1NeuriteLength" "D3NeuriteLength" "D5NeuriteLength"
> class(Concentration)
[1] "character"
> class(D1NeuriteLength)
[1] "numeric"
> class(D3NeuriteLength)
[1] "numeric"
> class(D5NeuriteLength)
[1] "numeric"

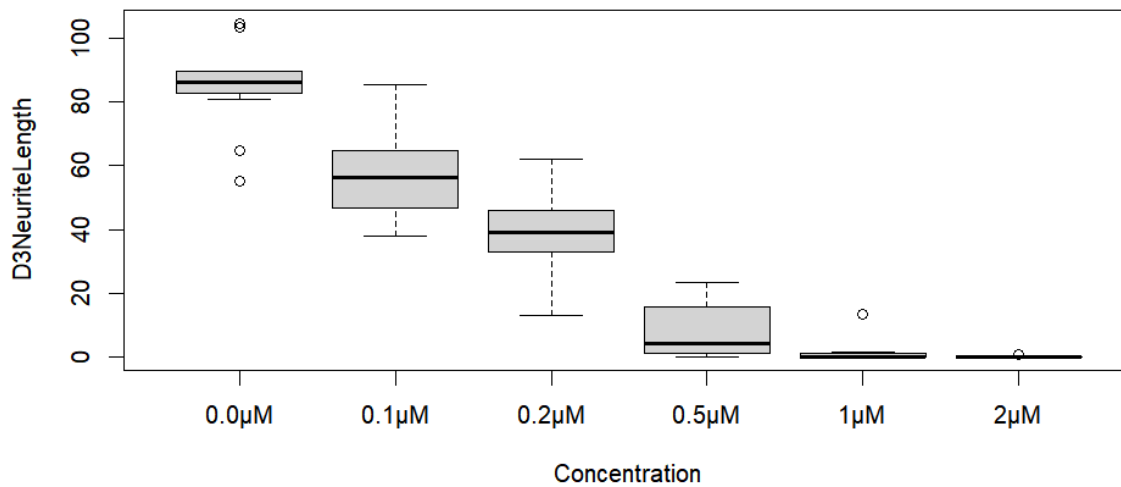
> factor(Concentration)
 [1] 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.0µM 0.1µM 0.1µM
[14] 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.1µM 0.2µM 0.2µM 0.2µM 0.2µM
[27] 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.2µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM
[40] 0.5µM 0.5µM 0.5µM 0.5µM 0.5µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM 1µM
[53] 1µM 1µM 1µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM 2µM
[66] 2µM
Levels: 0.0µM 0.1µM 0.2µM 0.5µM 1µM 2µM

> summary(NeuritesLength_by_dose_)
Concentration      D1NeuriteLength      D3NeuriteLength      D5NeuriteLength
Length:66          Min.   : 0.0000      Min.   : 0.0000      Min.   : 0.0000
Class :character   1st Qu.: 0.7218      1st Qu.: 0.5035      1st Qu.: 0.9337
Mode  :character   Median :16.1316      Median : 16.8028      Median : 20.3286
                    Mean  :24.3924      Mean  : 31.5770      Mean  : 25.5528
                    3rd Qu.:42.7449      3rd Qu.: 56.1183      3rd Qu.: 44.6875
                    Max.  :96.5118      Max.  :104.4726      Max.  :100.5488

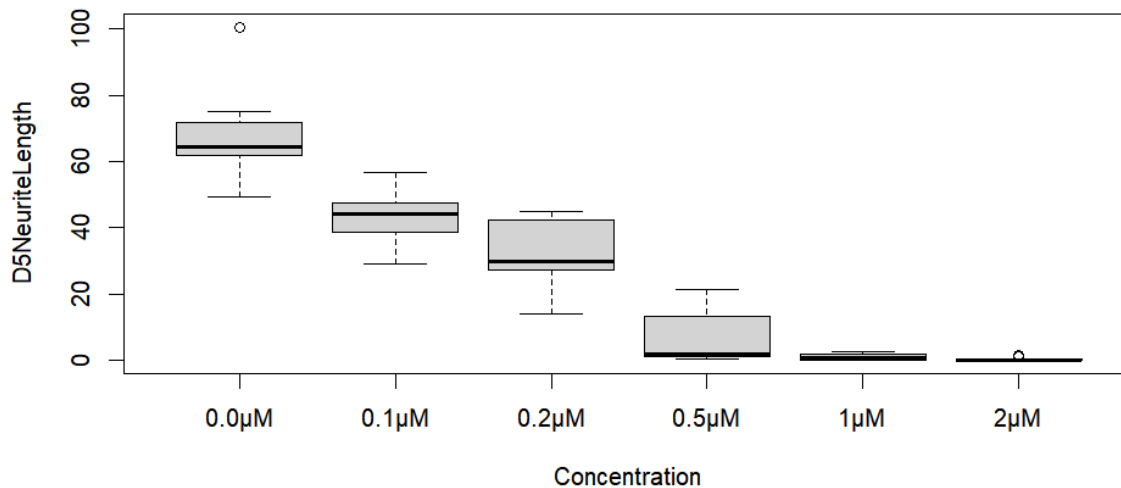
> boxplot(D1NeuriteLength~Concentration)
```



```
> boxplot(D3NeuriteLength~Concentration)
```



```
> boxplot(D5NeuriteLength~Concentration)
```



```
> aov(D1NeuriteLength~Concentration)
```

```
Call:  
aov(formula = D1NeuriteLength ~ Concentration)
```

Terms:

	Concentration	Residuals
Sum of Squares	43322.96	4319.14
Deg. of Freedom	5	60

Residual standard error: 8.484436
Estimated effects may be unbalanced

```
> aov(D3NeuriteLength~Concentration)
```

```
Call:
aov(formula = D3NeuriteLength ~ Concentration)
```

```
Terms:
```

	Concentration	Residuals
Sum of Squares	65248.80	6905.63
Deg. of Freedom	5	60

```
Residual standard error: 10.72818
Estimated effects may be unbalanced
```

```
> aov(D5NeuriteLength~Concentration)
```

```
Call:
aov(formula = D5NeuriteLength ~ Concentration)
```

```
Terms:
```

	Concentration	Residuals
Sum of Squares	41419.06	3969.16
Deg. of Freedom	5	60

```
Residual standard error: 8.133433
Estimated effects may be unbalanced
```

```
> AOV_D1_NL <- aov(D1NeuriteLength~Concentration)
```

```
> AOV_D3_NL <- aov(D3NeuriteLength~Concentration)
```

```
> AOV_D5_NL <- aov(D5NeuriteLength~Concentration)
```

```
> summary(AOV_D1_NL)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	5	43323	8665	120.4	<2e-16 ***
Residuals	60	4319	72		

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> summary(AOV_D3_NL)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	5	65249	13050	113.4	<2e-16 ***
Residuals	60	6906	115		

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> summary(AOV_D5_NL)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	5	41419	8284	125.2	<2e-16 ***
Residuals	60	3969	66		

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> TukeyHSD(AOV_D1_NL)
```

```
Tukey multiple comparisons of means
95% family-wise confidence level
```

```
Fit: aov(formula = D1NeuriteLength ~ Concentration)
```

```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-27.9586305	-38.60864	-17.308625	0.0000000
0.2µM-0.0µM	-40.7981764	-51.44818	-30.148171	0.0000000
0.5µM-0.0µM	-65.5137512	-76.16376	-54.863746	0.0000000
1µM-0.0µM	-69.0429465	-79.69295	-58.392941	0.0000000
2µM-0.0µM	-69.6572643	-80.30727	-59.007259	0.0000000
0.2µM-0.1µM	-12.8395459	-23.48955	-2.189541	0.0094360
0.5µM-0.1µM	-37.5551207	-48.20513	-26.905116	0.0000000
1µM-0.1µM	-41.0843160	-51.73432	-30.434311	0.0000000
2µM-0.1µM	-41.6986338	-52.34864	-31.048629	0.0000000
0.5µM-0.2µM	-24.7155748	-35.36558	-14.065570	0.0000001
1µM-0.2µM	-28.2447701	-38.89478	-17.594765	0.0000000
2µM-0.2µM	-28.8590879	-39.50909	-18.209083	0.0000000
1µM-0.5µM	-3.5291952	-14.17920	7.120810	0.9239297
2µM-0.5µM	-4.1435130	-14.79352	6.506492	0.8602876
2µM-1µM	-0.6143178	-11.26432	10.035687	0.9999793

```
> TukeyHSD(AOV_D3_NL)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = D3NeuriteLength ~ Concentration)
```

```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-27.561682	-41.02812	-14.095240	0.0000016
0.2µM-0.0µM	-46.791864	-60.25831	-33.325422	0.0000000
0.5µM-0.0µM	-76.456802	-89.92324	-62.990360	0.0000000
1µM-0.0µM	-82.973280	-96.43972	-69.506838	0.0000000
2µM-0.0µM	-84.439618	-97.90606	-70.973176	0.0000000
0.2µM-0.1µM	-19.230182	-32.69662	-5.763739	0.0011986
0.5µM-0.1µM	-48.895120	-62.36156	-35.428678	0.0000000
1µM-0.1µM	-55.411598	-68.87804	-41.945156	0.0000000
2µM-0.1µM	-56.877936	-70.34438	-43.411493	0.0000000
0.5µM-0.2µM	-29.664939	-43.13138	-16.198496	0.0000003
1µM-0.2µM	-36.181416	-49.64786	-22.714974	0.0000000
2µM-0.2µM	-37.647754	-51.11420	-24.181312	0.0000000
1µM-0.5µM	-6.516478	-19.98292	6.949965	0.7121526
2µM-0.5µM	-7.982815	-21.44926	5.483627	0.5083065
2µM-1µM	-1.466338	-14.93278	12.000105	0.9995263

```
> TukeyHSD(AOV_D5_NL)
```

```
Tukey multiple comparisons of means  
95% family-wise confidence level
```

```
Fit: aov(formula = D5NeuriteLength ~ Concentration)
```

```
$Concentration
```

	diff	lwr	upr	p adj
0.1µM-0.0µM	-24.2383197	-34.44773	-14.028907	0.0000000
0.2µM-0.0µM	-35.4232415	-45.63265	-25.213829	0.0000000
0.5µM-0.0µM	-60.6369276	-70.84634	-50.427515	0.0000000
1µM-0.0µM	-67.1477043	-77.35712	-56.938292	0.0000000
2µM-0.0µM	-67.7804434	-77.98986	-57.571031	0.0000000
0.2µM-0.1µM	-11.1849218	-21.39433	-0.975509	0.0238139
0.5µM-0.1µM	-36.3986078	-46.60802	-26.189195	0.0000000
1µM-0.1µM	-42.9093846	-53.11880	-32.699972	0.0000000
2µM-0.1µM	-43.5421237	-53.75154	-33.332711	0.0000000
0.5µM-0.2µM	-25.2136860	-35.42310	-15.004273	0.0000000
1µM-0.2µM	-31.7244628	-41.93388	-21.515050	0.0000000
2µM-0.2µM	-32.3572019	-42.56661	-22.147789	0.0000000
1µM-0.5µM	-6.5107768	-16.72019	3.698636	0.4258606
2µM-0.5µM	-7.1435159	-17.35293	3.065897	0.3221123
2µM-1µM	-0.6327391	-10.84215	9.576674	0.9999705

Entre días

```
> library(readxl)
> NeuritesLength_by_day_ <- read_excel("C:/Users/EVELIN CUADRO/Desktop/Datos MCZ Toxicity/NeuritesLength (by day).xlsx",
+   col_types = c("text", "numeric", "numeric",
+   "numeric", "numeric", "numeric",
+   "numeric"))

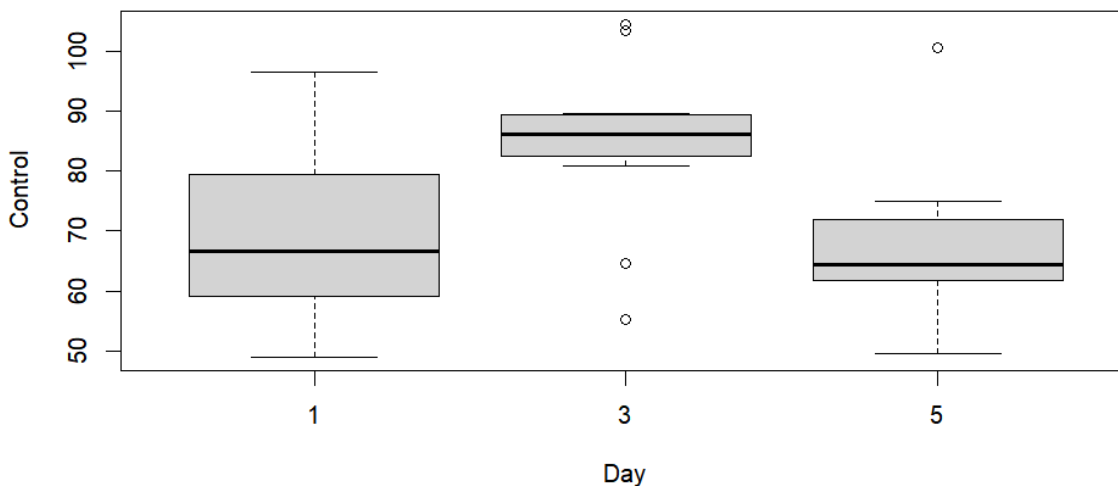
> view(NeuritesLength_by_day_)
> attach(NeuritesLength_by_day_)

> names(NeuritesLength_by_day_)
[1] "Day"      "Control" "C1"      "C2"      "C3"      "C4"      "C5"
> factor(Day)
 [1] 1 1 1 1 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3 3 3 3 3 5 5 5 5 5 5 5 5 5 5 5
Levels: 1 3 5
> class(Control)
[1] "numeric"
> class(Day)
[1] "character"
> class(C1)
[1] "numeric"
> class(C2)
[1] "numeric"
> class(C3)
[1] "numeric"
> class(C4)
[1] "numeric"
> class(C5)
[1] "numeric"

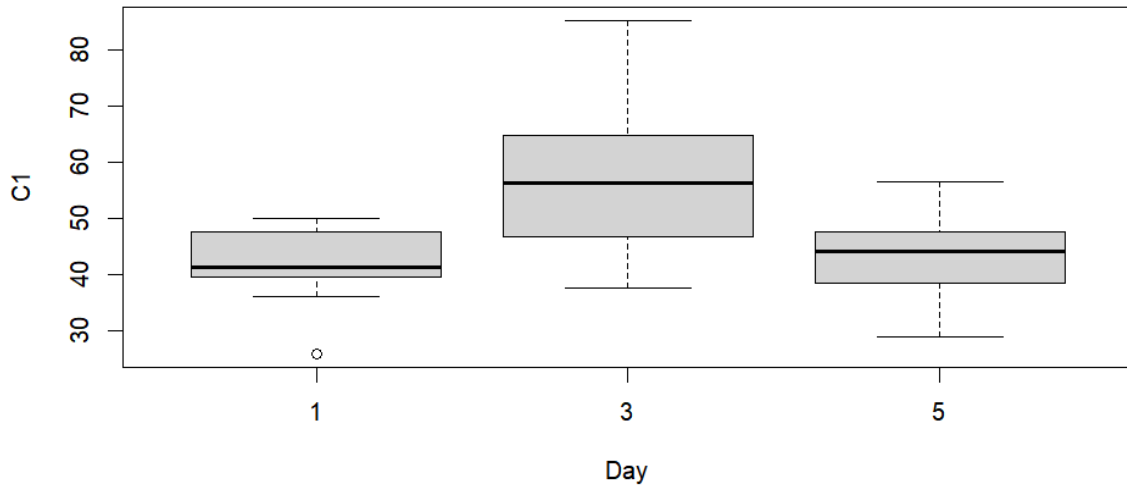
> summary(NeuritesLength_by_day_)
  Day      Control      C1      C2
Length:33   Min.   : 48.90   Min.   :25.93   Min.   :13.25
Class :character 1st Qu.: 62.31   1st Qu.:40.58   1st Qu.:25.23
Mode  :character Median : 69.04   Median :46.10   Median :32.79
      Mean  : 74.20   Mean  :47.61   Mean  :33.19
      3rd Qu.: 89.02   3rd Qu.:50.18   3rd Qu.:42.91
      Max.  :104.47   Max.  :85.30   Max.  :62.00

      C3      C4      C5
Min.   : 0.000   Min.   : 0.0000   Min.   :0.0000
1st Qu.: 1.463   1st Qu.: 0.0000   1st Qu.:0.0000
Median : 3.853   Median : 0.6454   Median :0.0000
Mean   : 6.662   Mean   : 1.1428   Mean   :0.2383
3rd Qu.: 9.242   3rd Qu.: 1.0523   3rd Qu.:0.2856
Max.   :23.260   Max.   :13.4835   Max.   :1.3788

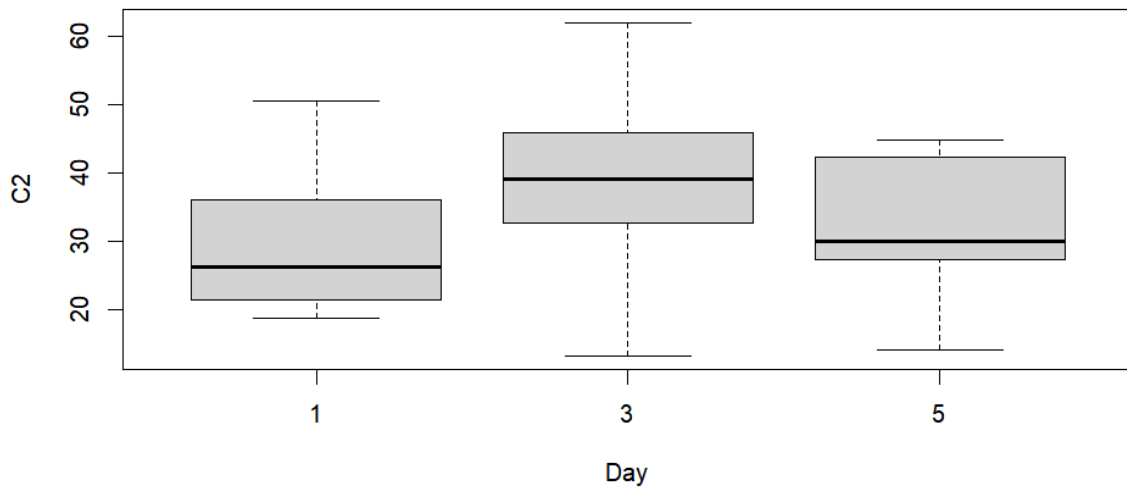
> boxplot(Control~Day)
```



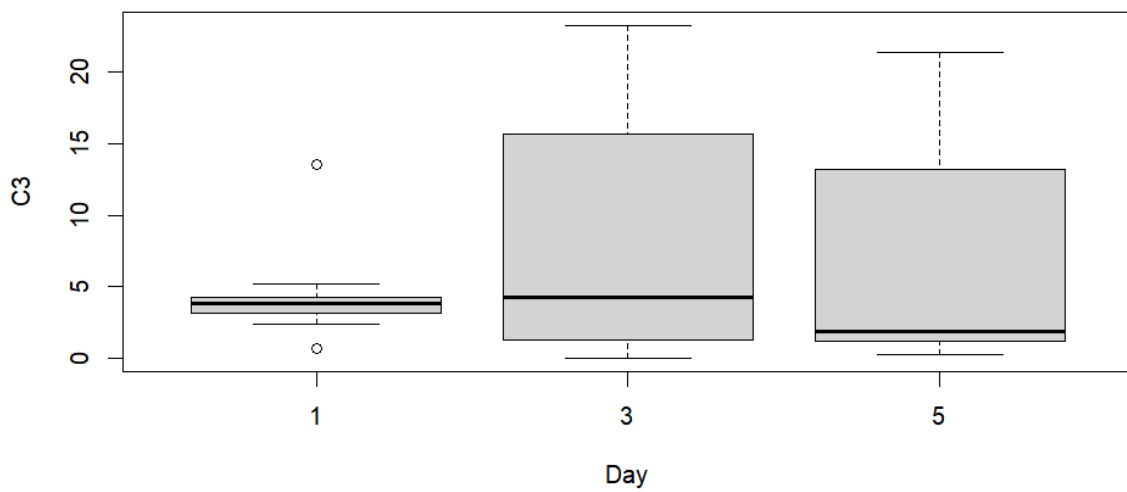
```
> boxplot(C1~Day)
```



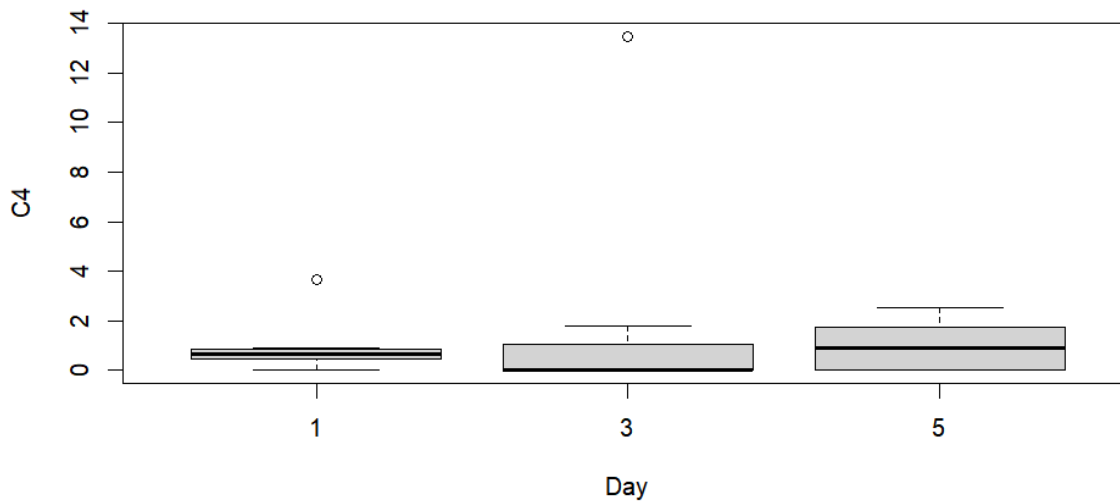
```
> boxplot(C2~Day)
```



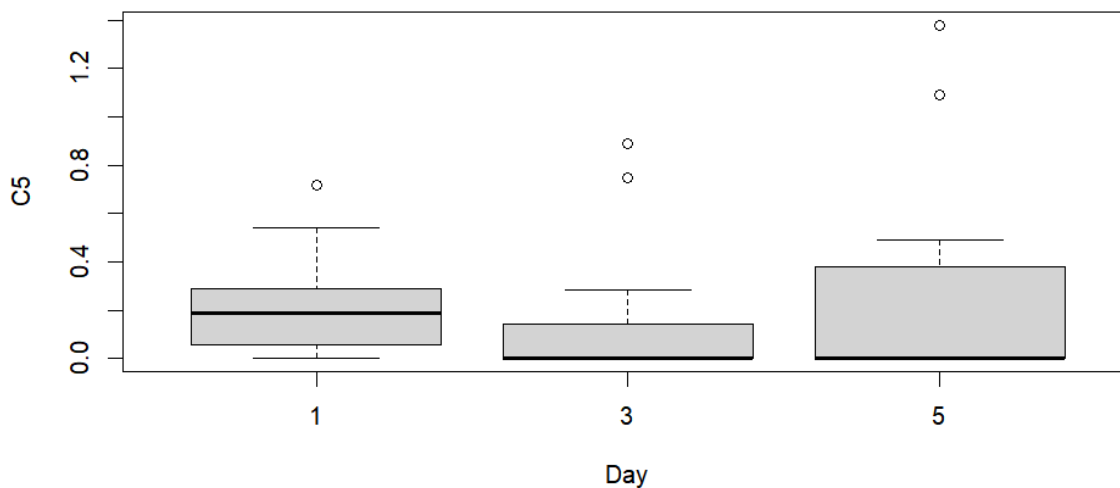
```
> boxplot(C3~Day)
```



```
> boxplot(C4~Day)
```



```
> boxplot(C5~Day)
```



```
> aov(Control~Day)
```

```
Call:
aov(formula = Control ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	1808.166	6412.784
Deg. of Freedom	2	30

Residual standard error: 14.62052
Estimated effects may be unbalanced

```
> aov(C1~Day)
```

```
Call:
aov(formula = C1 ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	1491.129	3130.605
Deg. of Freedom	2	30

Residual standard error: 10.21536
Estimated effects may be unbalanced

```
> aov(C2~Day)
```

```
Call:
  aov(formula = C2 ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	424.019	4000.873
Deg. of Freedom	2	30

Residual standard error: 11.54827
Estimated effects may be unbalanced

```
> aov(C3~Day)
```

```
Call:
  aov(formula = C3 ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	89.0875	1466.8404
Deg. of Freedom	2	30

Residual standard error: 6.992473
Estimated effects may be unbalanced

```
> aov(C4~Day)
```

```
Call:
  aov(formula = C4 ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	4.14761	178.83057
Deg. of Freedom	2	30

Residual standard error: 2.44152
Estimated effects may be unbalanced

```
> aov(C5~Day)
```

```
Call:
  aov(formula = C5 ~ Day)
```

Terms:

	Day	Residuals
Sum of Squares	0.102055	4.000958
Deg. of Freedom	2	30

Residual standard error: 0.3651921
Estimated effects may be unbalanced

```
> AOV_CONTROL_NL <- aov(Control~Day)
```

```
> AOV_C1_NL <- aov(C1~Day)
```

```
> AOV_C2_NL <- aov(C2~Day)
```

```
> AOV_C3_NL <- aov(C3~Day)
```

```
> AOV_C4_NL <- aov(C4~Day)
```

```
> AOV_C5_NL <- aov(C5~Day)
```

```
> summary(AOV_CONTROL_NL)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	1808	904.1	4.229	0.0241 *
Residuals	30	6413	213.8		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> summary(AOV_C1_NL)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	1491	745.6	7.145	0.0029 **
Residuals	30	3131	104.4		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> summary(AOV_C2_NL)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	424	212.0	1.59	0.221
Residuals	30	4001	133.4		

> summary(AOV_C3_NL)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	89.1	44.54	0.911	0.413
Residuals	30	1466.8	48.89		

> summary(AOV_C4_NL)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	4.15	2.074	0.348	0.709
Residuals	30	178.83	5.961		

> summary(AOV_C5_NL)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	2	0.102	0.05103	0.383	0.685
Residuals	30	4.001	0.13337		

MONTECARLO ANALYSIS

```
# Paso 1: Crear un marco de datos
```

```
set.seed(23)
```

```
#Simulación de Monte Carlo Adhesion Energy Axon 48 H
```

```
v1<-runif(50,0, 2227.541)
```

```
v2<-runif(50,0, 111.876)
```

```
v3<-runif(50,0, 1341930.588)
```

```
v4<-runif(50,0, 20812.306)
```

```
datos <- data.frame(
```

```
  Treatments = rep(c("0.0um", "0.5um", "1.0um", "2.5um"), each = 50),
```

```
  Valor = c(v1,v2,v3,v4
```

```
)
```

```
)
```

```
# Paso adicional: Calcular el valor absoluto de cada dato
```

```
datos$ValorAbsoluto <- abs(datos$Valor)
```

```
# Paso adicional: Expresar los valores absolutos como porcentajes del valor máximo
```

```
max_valor_absoluto <- max(datos$ValorAbsoluto)
```

```
datos$PorcentajeAbsoluto <- (datos$ValorAbsoluto / max_valor_absoluto) * 100
```

```
# Paso Adicional: Ordenar los niveles del factor para los tratamientos en el orden deseado
```

```
datos$Treatments <- factor(datos$Treatments, levels = c("0.0um", "0.5um", "1.0um", "2.5um"))
```

```
#Paso 2: Visualizar los valores absolutos para cada grupo en un boxplot
```

```
# Cargar la biblioteca ggplot2 si aún no está cargada
```

```
if (!requireNamespace("ggplot2", quietly = TRUE)) {
```

```
  install.packages("ggplot2")
```

```
}
```

```
library(ggplot2)
```

```
# Cargar la biblioteca RColorBrewer para utilizar la paleta "Set2"
```

```
if (!requireNamespace("RColorBrewer", quietly = TRUE)) {
```

```

install.packages("RColorBrewer")
}
library(RColorBrewer)
# Cambiar automáticamente el color por grupos y agregar cuadrícula
bp <- ggplot(datos, aes(x = Treatments, y = PorcentajeAbsoluto, fill = Treatments)) +
  geom_boxplot() +
  labs(title = "Adhesion Energy 48 H", x = "Treatments  $\mu\text{m}$ ", y = "Percentage of the Control") +
  scale_fill_brewer(palette = "Set3") + # Paleta de colores "Set3"
  theme_classic() +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    axis.text.x = element_text(face = "bold"),
    axis.title.x = element_text(face = "bold"),
    axis.text.y = element_text(face = "bold"),
    axis.title.y = element_text(face = "bold"),
    panel.grid.major = element_line(color = "gray", linetype = "dashed")
  ) #+
#scale_y_continuous(limits = c(30, 150)) # Establecer la escala del eje y de 30 a 150
# Mostrar el gráfico
print(bp)
# Paso 3: ANOVA
ajuste_anova <- aov(Valor ~ Treatments, data = datos)
summary(ajuste_anova)
# Paso 4: Dunnett's Test
library(DescTools)
# Realizar el test de Dunnett con "TN" como grupo de control
comparaciones_dunnett <- DunnettTest(x = datos$Valor, g = datos$Treatments, control = "0.0um")
print(comparaciones_dunnett)

```

RESULTADOS

NUCELEUS 24H

#Modulo Nucleus 24 H

v1<-runif(50,0, 2227.541)

v2<-runif(50,0, 111.876)

v3<-runif(50,0, 1341930.588)

v4<-runif(50,0, 20812.306)

diff lwr.ci upr.ci pval

0.5um-0.0um -1199.344 -88021.66 85622.97 1.0000

1.0um-0.0um 636304.777 549482.46 723127.09 <2e-16 ***

2.5um-0.0um 11063.546 -75758.77 97885.86 0.9815

#Stiffness Nucleus 24 H

v1<-runif(50,0,1772.774)

v2<-runif(50,0, 7925.025)

v3<-runif(50,0, 7624.378)

v4<-runif(50,0, 41657.809)

\$`0.0um`

diff lwr.ci upr.ci pval

0.5um-0.0um 3104.382 67.37959 6141.384 0.0437 *

1.0um-0.0um 2702.381 -334.62078 5739.383 0.0930 .

2.5um-0.0um 18285.694 15248.69171 21322.696 <2e-16 ***

#Disipation Nucleus 24 H

v1<-runif(50,0, 25,674)

v2<-runif(50,0, 1,883)

v3<-runif(50,0, 8,945)

v4<-runif(50,0, 30,932)

diff lwr.ci upr.ci pval

0.5um-0.0um -13.522908 -16.284563 -10.761253 < 2e-16 ***
1.0um-0.0um -10.226021 -12.987676 -7.464366 1.4e-15 ***
2.5um-0.0um 3.833843 1.072188 6.595498 0.0036 **

#Z Nucleus 24 H

v1<-runif(50,-162.142, 393.808)

v2<-runif(50,-118.006, 216.196)

v3<-runif(50,-124.845, 73.933)

v4<-runif(50,-209.33, 665.551)

diff lwr.ci upr.ci pval

0.5um-0.0um -100.1945 -175.0536 -25.33546 0.0050 **

1.0um-0.0um -181.7252 -256.5843 -106.86617 7.3e-08 ***

2.5um-0.0um 157.2206 82.3615 232.07964 6.0e-06 ***

#Deformation Nucleus 24 H

v1<-runif(50,0, 7.86)

v2<-runif(50,0, 20.133)

v3<-runif(50,0, 11.074)

v4<-runif(50,0, 7.197)

diff lwr.ci upr.ci pval

0.5um-0.0um 5.7571555 3.9812030 7.533108 9.8e-12 ***

1.0um-0.0um 0.8296069 -0.9463456 2.605559 0.5513

2.5um-0.0um -0.1715815 -1.9475339 1.604371 0.9917

#Adhesion Force Nucleus 24 H

v1<-runif(50,0, 133.605)

v2<-runif(50,0, 56.307)

v3<-runif(50,0.378, 127.736)

v4<-runif(50,0, 129.927)

```
diff lwr.ci upr.ci pval
0.5um-0.0um -46.835158 -62.34152 -31.328795 2e-10 ***
1.0um-0.0um -14.444248 -29.95061 1.062115 0.0741 .
2.5um-0.0um 1.577247 -13.92912 17.083610 0.9904
```

#Adhesion Energy Nucleus 24 H

```
v1<-runif(50,0, 10.475)
```

```
v2<-runif(50,0, 1.880)
```

```
v3<-runif(50,0, 8.971)
```

```
v4<-runif(50,0, 10.036)
```

```
diff lwr.ci upr.ci pval
0.5um-0.0um -4.95472479 -6.094822 -3.8146280 <2e-16 ***
1.0um-0.0um -1.64396722 -2.784064 -0.5038704 0.0022 **
2.5um-0.0um 0.03449477 -1.105602 1.1745915 0.9997
```

AXON 24 H

#Modulo Axon 24 H

```
v1<-runif(50,0,4355.980)
```

```
v2<-runif(50,0, 3110.809)
```

```
v3<-runif(50,0, 12303.900)
```

```
v4<-runif(50,0, 9782.487)
```

```
diff lwr.ci upr.ci pval
0.5um-0.0um -881.7317 -1978.899 215.4358 0.1448
1.0um-0.0um 3389.6239 2292.456 4486.7915 8.6e-12 ***
2.5um-0.0um 3334.5386 2237.371 4431.7061 5.4e-11 ***
```

#Stiffness Axon 24 H

```
v1<-runif(50,0,671.324)
```

```
v2<-runif(50,0, 255.115)
v3<-runif(50,0, 72645.812)
v4<-runif(50,0, 52995.406)
      diff lwr.ci upr.ci pval
0.5um-0.0um -249.4065 -6153.784 5654.971 0.9993
1.0um-0.0um 34136.0257 28231.648 40040.403 <2e-16 ***
2.5um-0.0um 30991.2578 25086.880 36895.636 <2e-16 ***
```

#Disipation Axon 24 H

```
v1<-runif(50,0, 29.859)
v2<-runif(50,0, 6.967)
v3<-runif(50,0, 8.403)
v4<-runif(50,0, 30.288)
      diff lwr.ci upr.ci pval
0.5um-0.0um -13.309645 -16.254937 -10.364353 <2e-16 ***
1.0um-0.0um -12.843171 -15.788464 -9.897879 <2e-16 ***
2.5um-0.0um 1.092995 -1.852297 4.038288 0.7098
```

#Z Axon 24 H

```
v1<-runif(50,-108.725, 508.9)
v2<-runif(50,-111.372, 154.513)
v3<-runif(50,-176.177, 94.135)
v4<-runif(50,-59.647, 530.289)
      diff lwr.ci upr.ci pval
0.5um-0.0um -216.32573 -279.18394 -153.4675 5.1e-13 ***
1.0um-0.0um -287.26234 -350.12055 -224.4041 < 2e-16 ***
2.5um-0.0um 50.04357 -12.81464 112.9018 0.1503
```

#Deformation Axon 24 H

v1<-runif(50,0, 4.744)

v2<-runif(50,0,15.021)

v3<-runif(50,0, 10.743)

v4<-runif(50,0, 5.666)

diff lwr.ci upr.ci pval

0.5um-0.0um 4.9269849 3.5430816 6.310888 8e-14 ***

1.0um-0.0um 2.4292507 1.0453473 3.813154 0.00013 ***

2.5um-0.0um 0.6790722 -0.7048312 2.062976 0.51312

#Adhesion Force Axon 24 H

v1<-runif(50,0, 130.223)

v2<-runif(50,0.332, 114.687)

v3<-runif(50,1.243, 124.960)

v4<-runif(50,0, 127.173)

diff lwr.ci upr.ci pval

0.5um-0.0um -15.219411 -31.99851 1.559689 0.0847 .

1.0um-0.0um -13.402229 -30.18133 3.376872 0.1483

2.5um-0.0um 1.853945 -14.92516 18.633046 0.9877

#Adhesion Energy Axon 24 H

v1<-runif(50,0, 10.466)

v2<-runif(50,0.001, 6.995)

v3<-runif(50,0.009, 8.391)

v4<-runif(50,0, 9.793)

diff lwr.ci upr.ci pval

0.5um-0.0um -2.3605633 -3.574205 -1.146921 2.6e-05 ***

1.0um-0.0um -1.9097308 -3.123373 -0.696089 0.00072 ***
2.5um-0.0um -0.1042706 -1.317912 1.109371 0.99412

NUCLEO 48 H

#Modulo Nucleus48 H

v1<-runif(50,0,102866.354)

v2<-runif(50,0, 370598.199)

v3<-runif(50,0, 234837.269)

v4<-runif(50,0, 8546.546)

diff lwr.ci upr.ci pval

0.5um-0.0um 129552.43 97926.24 161178.61 < 2e-16 ***

1.0um-0.0um 53573.38 21947.20 85199.57 0.00024 ***

2.5um-0.0um -52940.48 -84566.67 -21314.29 0.00036 ***

#Stiffness Nucleus48 H

v1<-runif(50,0, 11759.372)

v2<-runif(50,0, 1710.795)

v3<-runif(50,0, 42270.922)

v4<-runif(50,0, 25635.123)

diff lwr.ci upr.ci pval

0.5um-0.0um -5764.527 -9095.438 -2433.617 0.00018 ***

1.0um-0.0um 13452.890 10121.980 16783.801 < 2e-16 ***

2.5um-0.0um 8543.971 5213.061 11874.882 3e-08 ***

#Disipation Nucleus48 H

v1<-runif(50,0, 31.961)

v2<-runif(50,0, 32.701)

v3<-runif(50,0, 30.965)

```
v4<-runif(50,0, 26.435)
```

```
diff lwr.ci upr.ci pval
```

```
0.5um-0.0um -1.471390 -5.635711 2.6929305 0.7383
```

```
1.0um-0.0um -3.308982 -7.473303 0.8553386 0.1513
```

```
2.5um-0.0um -2.372903 -6.537224 1.7914178 0.3930
```

```
#Z Nucleus48 H
```

```
v1<-runif(50,-102.851, 521.339)
```

```
v2<-runif(50,-105.832, 613.62)
```

```
v3<-runif(50,-310.482, 508.62)
```

```
v4<-runif(50,-211.768, 405.235)
```

```
diff lwr.ci upr.ci pval
```

```
0.5um-0.0um 9.179352 -85.62513 103.9838340 0.9917
```

```
1.0um-0.0um -170.408886 -265.21337 -75.6044039 8.9e-05 ***
```

```
2.5um-0.0um -95.631119 -190.43560 -0.8266367 0.0476 *
```

```
#Deformation Nucleus48 H
```

```
v1<-runif(50,0, 4.797)
```

```
v2<-runif(50,0, 3.767)
```

```
v3<-runif(50,0, 46.564)
```

```
v4<-runif(50,0, 13.524)
```

```
diff lwr.ci upr.ci pval
```

```
0.5um-0.0um -0.3458803 -3.7461647 3.054404 0.9904
```

```
1.0um-0.0um 19.2816111 15.8813267 22.681895 <2e-16 ***
```

```
2.5um-0.0um 3.9918265 0.5915421 7.392111 0.0165 *
```

```
#Adhesion Force Nucleus48 H
```

```
v1<-runif(50,0, 134.792)
```

v2<-runif(50,0, 134.705)

v3<-runif(50,0, 134.494)

v4<-runif(50,0, 120.554)

diff lwr.ci upr.ci pval

0.5um-0.0um -7.828862 -25.65423 9.996510 0.5973

1.0um-0.0um -12.101154 -29.92653 5.724218 0.2561

2.5um-0.0um -4.640218 -22.46559 13.185154 0.8714

#Adhesion Energy Nucleus48 H

v1<-runif(50,0, 10.006)

v2<-runif(50,0, 9.888)

v3<-runif(50,0, 9.552)

v4<-runif(50,0, 9.051)

diff lwr.ci upr.ci pval

0.5um-0.0um -0.6376080 -1.946159 0.6709433 0.5186

1.0um-0.0um -1.1034922 -2.412043 0.2050590 0.1187

2.5um-0.0um -0.2841223 -1.592673 1.0244290 0.9192

AXON 48 H

#Modulo Axon 48 H

v1<-runif(50,0,11575.297)

v2<-runif(50,0, 13905.700)

v3<-runif(50,0, 640075.352)

v4<-runif(50,0, 37881.792)

```
diff lwr.ci upr.ci pval
0.5um-0.0um 510.8431 -40994.42 42016.11 1.0000
1.0um-0.0um 297577.8017 256072.54 339083.07 <2e-16 ***
2.5um-0.0um 15896.9765 -25608.29 57402.24 0.6904
```

#Stiffness Axon 48 H

```
v1<-runif(50,0,671.324)
v2<-runif(50,0, 255.115)
v3<-runif(50,0, 72645.812)
v4<-runif(50,0, 52995.406)
```

```
diff lwr.ci upr.ci pval
0.5um-0.0um -249.4065 -6153.784 5654.971 0.9993
1.0um-0.0um 34136.0257 28231.648 40040.403 <2e-16 ***
2.5um-0.0um 30991.2578 25086.880 36895.636 <2e-16 ***
```

#Disipation Axon 48 H

```
v1<-runif(50,0, 32.353)
v2<-runif(50,0, 9.525)
v3<-runif(50,0, 32.55)
v4<-runif(50,0, 10.056)
```

```
diff lwr.ci upr.ci pval
0.5um-0.0um -13.42130 -16.585197 -10.2573938 < 2e-16 ***
1.0um-0.0um -2.77696 -5.940862 0.3869415 0.0993 .
2.5um-0.0um -12.28921 -15.453111 -9.1253080 2.2e-16 ***
```

#Z Axon 48 H

```
v1<-runif(50,-59.252, 562.52)
v2<-runif(50,-50.736, 152.333)
v3<-runif(50,-71.234, 572.373)
```

```
v4<-runif(50,-99.151, 83.09)
      diff lwr.ci upr.ci pval
0.5um-0.0um -239.29079 -301.0559 -177.525635 7.8e-16 ***
1.0um-0.0um -56.77546 -118.5406 4.989698 0.0793 .
2.5um-0.0um -282.60010 -344.3653 -220.834942 < 2e-16 ***
```

#Deformation Axon 48 H

```
v1<-runif(50,0, 6.624)
v2<-runif(50,0, 6.197)
v3<-runif(50,0, 8.915)
v4<-runif(50,0, 8.14)
      diff lwr.ci upr.ci pval
0.5um-0.0um -0.5986609 -1.61373732 0.4164156 0.3652
1.0um-0.0um 0.5007485 -0.51432794 1.5158249 0.5089
2.5um-0.0um 1.0835100 0.06843355 2.0985864 0.0333 *
```

#Adhesion Force Axon 48 H

```
v1<-runif(50,0, 134.661)
v2<-runif(50,0, 134.613)
v3<-runif(50,0, 134.559)
v4<-runif(50,0, 115.507)
      diff lwr.ci upr.ci pval
0.5um-0.0um -7.801559 -25.46824 9.865121 0.5933
1.0um-0.0um -11.996410 -29.66309 5.670271 0.2560
2.5um-0.0um -7.553846 -25.22053 10.112834 0.6166
```

#Adhesion Energy Axon 48 H

```
v1<-runif(50,0, 10.098)
v2<-runif(50,0, 9.511)
```

```
v3<-runif(50,0, 10.040)
```

```
v4<-runif(50,0, 8.081)
```

```
diff lwr.ci upr.ci pval
```

```
0.5um-0.0um -0.8802723 -2.163424 0.4028789 0.2484
```

```
1.0um-0.0um -0.9235126 -2.206664 0.3596386 0.2145
```

```
2.5um-0.0um -0.9101707 -2.193322 0.3729805 0.2247
```