## 1.LOGIT METHOD

To calculate fractions: (table 1 in excel example)
Number of all living specimens or total fresh biomass, length of root, shoot in the control divide per your result For example:
Average number of shoot length $=0.85$, in the control $=8.5$ ( for ex. for concentration 50\%)
Fraction $(\mathrm{F})=0.85 / 8.5$ if the result is more than 1 you should do additionally subtraction like this: $(1-$ your result)
2. To calculate $\operatorname{logit}(\mathrm{Y}): \mathrm{y}=\log (\mathrm{F} / 1-\mathrm{F})$
$\mathrm{F}=$ fraction
( table 2 in excel example)
You should also calculate $\log$ from concentrations ( $\log \mathrm{C}$ )
3 Then you make a graph (chart)
Axis $\mathrm{X}=\log \mathrm{c}$
Axis $\mathrm{Y}=\log (\mathrm{F} / 1-\mathrm{F})$
4. You should add trend line and regression formula/ equation (double click on trend line)

It should be like this : $\mathrm{y}=1.5 \mathrm{x}-1.2$
$\mathrm{R}^{2}=0.87(\mathrm{R}$ should be $>0.5)$
1.5 in our example is $B$ value
1.2 in our example is A value so general equation would be $\mathrm{Y}=\mathrm{Bx}-\mathrm{A}$
5. then you should calculate IC (inhibition concentration)
$\mathrm{IC} / \mathrm{LC}_{50}=((\log \mathrm{P} / 1-\mathrm{P})-\mathrm{A}) / \mathrm{B}$
P for IC/LC $50=0.5$
So after simplification
$\log \left(\mathrm{IC} / \mathrm{LC}_{50}\right)=-(\mathrm{A}) / \mathrm{B}$
you could also calculate $\mathrm{IC}_{20}$ it depends on your results for $\mathrm{IC}_{20} \mathrm{P}=0.2$
the results are in the last two lines in the table 2 in the excel example, finally you calculate IC/LC 50 or IC/LC 20 not $\log \mathrm{IC} / \mathrm{LC}{ }_{50}$ or $\log \mathrm{IC} / \mathrm{LC}_{20}$ ( last line of table 2)
You should calculate IC/LC separately for length of shoot, root, fresh biomass
good luck !!!!
Example:
Table

| control | 0,31 | 0,62 | 1,25 | 2,5 | 5 | 10 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 10 | 9 | 7 | 5 | 3 | 2 | 0 |
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|  |  |  |  |  |  |  |
| 10 | 9 | 7 | 5 | 3 | 2 | 0 |
|  |  |  |  |  |  |  |
|  | 0,9 | 0,7 | 0,5 | 0,3 | 0,2 | 0 |


| $\log C$ | Logits |
| :---: | :---: |
|  | Rejected |
|  | 1 value (R) |

$0,70 \quad 0,60205999$
$0,40 \quad 0,36797679$
$0,10 \quad 0$
-0,21 0,36797679
$-0,51 \quad 0,95424251$


Results $\mathrm{y}=\mathrm{Bx}-\mathrm{a}$

- 0,1921/-1,2741

| $\log c$ | 0,15077309 |
| :--- | :--- |
| LC 50 | 1,41505427 |

## 2 REED METHOD

Table 1. The example of results for the calculation of $\mathrm{LC}_{50}$ by the Reed method.

| Concentration$\mathrm{mg} / \mathrm{dm}^{3}$ | Number of animals |  | Number of animals after cumulation |  |  | $\begin{gathered} \text { Percentage of } \\ \text { mortality } \\ \mathrm{P}=\mathrm{m} \cdot 100 / \mathrm{b} \\ \hline \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | dead | alive | dead | alive | studied |  |
| 1.0 | 2 | 8 | 2 | 10 | 12 | 16.6 |
| 2.0 | 8 | 2 | 10 | 2 | 12 | 83.33 |
| 4.0 | 10 | 0 | 20 | 0 | 20 | 100 |

Calculation of $\mathrm{LC}_{50}: \log \mathrm{LC}_{50}=\log \mathrm{x}+\mathrm{k} \cdot \log \mathrm{i}$
$\mathrm{k}=(50-\mathrm{P} 1) /(\mathrm{P} 2-\mathrm{P} 1)$
$x$ - concentration causing the nearest $50 \%$ of mortality
i - quotient of geometric progression (1.1 ... 2.0)
k -coefficient
P1 -cumulative \% of mortality lower than 50\%
P 2 - cumulative \% of mortality higher than $50 \%$

