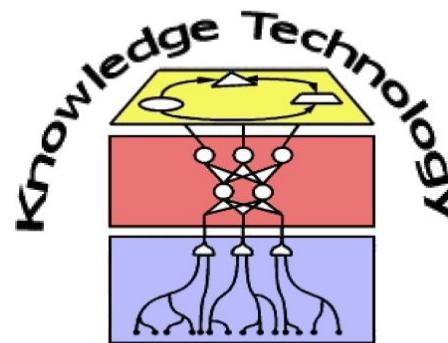


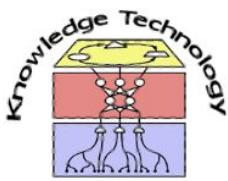
Research Methods

Assignment 7: Research Paper Review

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Outline

Long term Toxicity of Roundup herbicide and a Roundup tolerant GM Maize

- Overall Study
- Hypotheses
- Experiment Design
 - Experiment Design: Findings
- Statistical Approach
 - Statistical Approach: Findings
- Experiment Analysis and Conclusion
- General Review

Long-term toxicity of a Roundup herbicide and a Roundup-tolerant GM maize

Roundup containing glyphosate herbicide has been widely used for decades to make plants weed resistant. Despite the increase in harvest, many people claim that spraying crops with Roundup leads to cancer.

Seralini et al. conducted a 2 years study on rats to see if there is a health effect from Roundup or GM maize.



Hypotheses

- H_0 There is an evidence of a long-term toxicity from Roundup or GM maize.
- H_1 Roundup and GM maize has no effect on health.

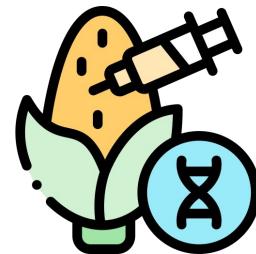


Experiment Design

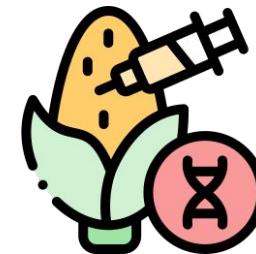
- Monsanto tested 2 doses of Roundup and GM maize for 90 days on rats → no effect on health
- Experiment assumes 2 years study will show long-term toxicity of Roundup or GM maize
- **10 treatment groups** tested on Sprague-Dawley strain rats:



Control
(plain water and
non-GM maize)



GM maize (11%, 22%, 33%)



**GM maize (11%, 22%, 33%)
+ Roundup**



Roundup
(A - 40mg, B - 200mg,
C - 2.5g)

Experiment Design: Findings

- **Insufficient number of rats per treatment group:**
 - Experiment was designed as chronic disease study (10 rats per gender in group) and not carcinogenic study (50 rats per gender in group).
 - So researchers has almost no evidence to state that tumors were caused by Roundup or GM maize treatment. generated.
- **No information on diet and water in the experiment:**
 - No details on how much food each rat consumed → could be the reason why tumors progressed unproportionally to treatment
 - No information if water used for feeding was without pesticides and the premises
- **Inadequate Data presented in the study:**
 - Researchers emphasize on the fact that all data cannot be presented.
 - Unsure about the validity of the results because of lack of information and statistical analysis.



Experiment Design: Findings

- What is the **control group**?
 - No information on a treatment received by control group.
- **Rat characteristics not taken into account:**
 - Sprague-Dawley strain rat females have high tendency of mammary tumors [6].
 - Tumours observed in experiment may not have treatment as origin.
- **Animal welfare issues:**
 - Animals were not euthanized in time in accordance with European legislation on laboratory animal protection.
 - 2 Year study is considered irrelevant as around 70-80% of the rats develop tumours under normal conditions [2, 3, 4]. Therefore, it could have been restricted to ~90 days.
- **Tumours:**
 - No images for tumors in control group are shown.
 - No frequency analysis for the tumour is present between the groups.
 - Early death of rats because of tumours is common and could not be related to the treatment [1].



Statistical Approach

- **Independent Variables:**
 - Diet
- **Dependent Variables:**
 - Tumor progression
 - Liver and Kidney diseases
- **Controlled Variables:**
 - Rat species
 - Age of rats
 - Conditions in which rats were kept
 - Amount of rats per group



Statistical Approach: Findings

- **Independent Variables** related to rats that were not considered:
 - Weights of the rats (randomly distributed based on weights)
 - Health parameters for every rat (at 5 weeks of age)
 - Acclimatization of rats (ability to adapt to environmental changes)
- **Extraneous Factors:** These are the factors which could have affected the overall experiment and were not considered before conducting the experiment:
 - Sprague-Dawley strain rats pathologies
 - Rats were kept in cages and not in their natural habitat
 - Diet description doesn't mention if all rats had limited access to food and water. This could affect why tumors developed not proportionally to treatment
 - Various pesticides and premises presented in the water



Statistical Approach: Findings

However,

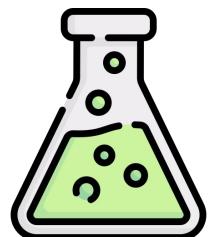
- There is no specific information provided related to the statistical analysis (ARRIVE guidelines)
- The study is not subjected to standard statistical analysis
- Complete data for the study could not be found.
- **Blinding:**
 - Treatment allocation should have been concealed but was not.
 - This could be a relevant source of bias.
- **Sample Size:**
 - To achieve significant statistical power, the sample size is too small.
 - How this size was calculated (ARRIVE guidelines), is not mentioned in the paper.
- **False Claims:**
 - It has been found in various findings that the female mortality rate reported is false and accounts for much less.
 - Similarly, no evidence has been found for the claim made by the researchers [5].



Experiment Analysis and Conclusion

for calculating the mortality rates Chi-square could have been used which is an easier method

- To calculate **mortality rates** researchers should have used **Chi-square** test of Brandt and Snedecor for $2 \times k$ contingency tables.
- **No proper background at pathologies:**
 - The researchers were unaware of spontaneous pathologies of Sprague-Dawley strain rats.
 - Wilm's tumors which was the reason for euthanasia in most of the rats is a spontaneous tumor that may occur in a life span.
- **Uncommon statistical tests** (Kruskal-Wallis, Dunn's, Westlake exceedance, Kolmogorov-Smirnov, Wilcoxon-Mann-Whitney):
 - Should use robust statistical tests to determine statistical significant difference.
- The paper was finally retracted by the publisher because of inconsistencies in the paper.



General review

- Conducting the experiment with more doses (3 compared to 2 from Monsanto's studies).
- Control groups helped them compare the interaction effects.



General review

- Paper doesn't provide sufficient information in which conditions rats were kept.
- As well as, what were the diets and how the food was prepared.
- The paper doesn't provide any information on how the tumors progressed and their stages.
- Researchers made animals suffer by allowing tumors to develop till 25% of rat's body, which is considered very unethical.
- Researchers didn't share complete data with other scientists as is required by Elsevier's published policies.



Thank you for your attention.



Literature:

- [1] Son, Woo-Chan, et al. "Profile of early occurring spontaneous tumors in Han Wistar rats." *Toxicologic pathology* 38.2 (2010): 292-296.
- [2] Kilkenny, Carol, et al. "Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research." *PLoS biol* 8.6 (2010): e1000412.
- [3] Suzuki, H., U. Mohr, and G. Kimmerle. "Spontaneous endocrine tumors in Sprague-Dawley rats." *Journal of cancer research and clinical oncology* 95.2 (1979): 187-196.
- [4] Prejean, J. D., et al. "Spontaneous tumors in Sprague-Dawley rats and Swiss mice." *Cancer research* 33.11 (1973): 2768-2773.
- [5] Séralini, Gilles-Eric, et al. "RETRACTED: Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize." (2012): 4221-4231.
- [6] Prejean, J. D., et al. "Spontaneous tumors in Sprague-Dawley rats and Swiss mice." *Cancer research* 33.11 (1973): 2768-2773.