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# A comparison of statistical methods to establish no effect

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# Abstract

Various statistical methods are designed or used to establish 'no effect'. The true absence of an effect is not necessarily confirmed by a non-significant ( $p\text{-value} \geq 0.05$ ) result of a traditional null hypothesis significance test (NHST). This is due to various factors such as too small effect sizes, a lack of statistical power or p-value associated Type I and Type II error rates. In a similar way to the concept of error rates, a 'false trust rate' is the rate at which a statistical method suggests trust in a non-significant result despite the presence of a real effect, and a 'false mistrust rate' is the rate at which a statistical method falsely suggests mistrust of a non-significant result. These rates vary among statistical approaches, like the minimum detectable difference (MDD), Confidence Intervals (CI), Bayes factors (BF) or Equivalence test (EQUIV). They can be used to establish no effect concentrations of harmful chemicals, thereby ensuring the safety of human health and the environment. To prevent environmental damage, potentially toxic chemicals are regulated through legislation by policymakers and the expertise of scientists. Scientists identify potentially toxic chemicals and their potential harmful effects by above mentioned statistical methods. However, the determination of the most appropriate statistical method remains an important decision in establishing guidelines for risk assessment. In this study, I compare the four techniques in terms of their False Mistrust and False Trust Rates (FMR and FTR), to reflect the probability of detecting true negatives among non-significant results when using each approach. I explain the theory behind the methods, including the concept of MDD and CIs, the Bayesian approach using BF, and the principles of EQUIV to provide a comprehensive understanding. I perform simulations based on the R script by Mair et al. (2020) and I will look at the European Food Safety Authority's (EFSA) switch to the use of EQUIV and associated consequences for practical application. In practice it is a balancing act to choose between statistical methods that provide reliable information on whether a concentration is acceptable or not, and to find a method that saves resources in terms of the number of animals tested. My main results are that CIs and EQUIV are performing the same in identifying true effects if they are present and that the new EFSA guidelines with the use of EQUIV require more animal testing than in the past. In conclusion, I support the findings of Mair et al. (2020) in recommending the use of CIs for identifying true negatives among non-significant results and therefore for establishing no effect.

# 1. Introduction

To establish the true absence of an effect, it is necessary to understand the interpretation of non-significant ( $p\text{-value} \geq 0.05$ ) results. A non-significant result of an NHST can mean that there is either evidence for the null hypothesis, indicating that no difference between parameters of groups exists, or that the data is insensitive to find any differences (Dienes, 2014). The insensitivity of the data depends on effect size and sample size. In a large sample, smallest effects of no importance can be found to be significant, whereas in a small sample, even large effects of relevance may be found non-significant (Faller, 2004). The sample size determines both the dispersion of the data around the mean, and therefore the variance, and the ability of a test to detect a significant effect when it is present, and thus the statistical power (Satterthwaite, 1941, Fay, 2007). The power is the complementary event of the Type II error, which is equivalent to a false-negative result. A false-negative result occurs when the null hypothesis is not rejected, although there is an effect that results in differences between the groups' parameters (Kaur, 2017). In addition to the Type II error, there is another error that can interfere with hypothesis testing, the Type I error. This error corresponds to a false positive, which results when the null hypothesis is incorrectly rejected even though there is no effect (Newman, 2008). In a similar way to the concept of error rates, a 'false trust rate' (FTR) is the rate at which a statistical method suggests trust in a non-significant result despite the presence of a real effect, and a 'false mistrust rate' (FMR) is the rate at which a statistical method falsely suggests mistrust of a non-significant result (Mair, 2020). These FTR and FMR illustrate the precision identifying true negatives among non-significant results and vary among methods for detecting no effects.

Detecting effects of potentially harmful chemicals is crucial because chemicals are an integral part of modern industry. Since industrial revolution, chemicals have been used extensively in a wide range of industries such as textiles, food, cosmetics, or agricultural production (Heaton, 1993). Agricultural crops are protected from damage using pesticides, which control pests like insects, weeds, or fungi through their chemical activity (Mahmood 2016). The chemical effects on non-target organisms have been studied over years for certain pesticides and lead to increasing evidence that the use of pesticides is associated with serious health and environmental risks (Williams, C. M. 1967, Nesheim, 1978, Ware, 1980, van der Werf, 1996, Fenik, 2011, Carvalho, 2017, Aguilar-Marcelino, 2023). The environmental and health damage caused by direct or indirect exposure to chemicals leads to habitat changes and affects biodiversity (Isenring, R., 2010). Since the adverse ecological side-effects of authorized chemicals became known, public awareness and concern have led policymakers to commission scientists who monitor the potentially harmful effects of chemicals.

Scientists identify the fate and effects of potentially toxic chemicals in ecosystems and advise policymakers on appropriate regulation of chemicals in the environment (Kendall, R., 2001). An example of proper risk assessment is the European Union's REACH regulation. 'Registration, Evaluation, Authorization and Restriction of Chemicals' (REACH) was introduced in 2006 and aims to protect human health and the environment from the risks posed by potentially harmful effects of chemicals (Williams, 2011). In addition to REACH, which

regulates the authorization and evaluation of industrial chemicals to be placed on the European market, there is the European Food Safety Authority (EFSA). EFSA is an impartial agency of the European Union that provides scientific advice on environmental risk assessment, legislation, and regulations to protect human health and the environment from food-related risks (see <https://www.efsa.europa.eu/en/about/about-efsa>, Oltmanns, 2019). The legislation and scientific advice on the fate and effects of potentially toxic chemicals in the environment rely on meaningful statistical approaches.

The most precise statistical method for ecotoxicological risk assessment is still widely debated. Historically, ecotoxicological risk assessment studies have used the no-observed-effect level (NOEL) or no-effect concentration (NEC) to establish a threshold at which adverse effects occur. The highest dose that does not cause a toxic effect (NOEL) is determined by significant differences between a treatment and control group using hypothesis testing (e.g. Wang, 1988, Srivastava, 1999, EFSA, 2016, EFSA 2017, EFSA, 2019). Because the name NOEL is considered misleading, scientists refer to the highest dose that does not cause a toxic effect as the No Observed Effect Concentration (NOEC) (Warne, 2008). The use of the NOEC is controversial: Skalski (1981) argued that NOECs violate the principle of negative inference. Hoekstra (1993) criticized that the use of NOECs is associated with a severe power problem. To overcome the problem of too low power, a statistical analysis for the concentration-response relationship determined by NOEC can be performed using minimum detectable differences (MDD) (Brock, 2015, EFSA, 2016, EFSA 2017, EFSA, 2019). In addition to MDD, other statistical methods could be used to determine no-effect concentrations: The Confidence Interval (CI) is an interval estimate for a certain parameter of interest (Le, 2003) and the location of its boundaries relative to a predetermined threshold can be used to predict whether an effect is 'equivalent to zero' or not (Lakens, 2017). For establishing an 'acceptable low' or 'equivalent to zero' effect concentration, equivalence tests, which test whether the differences between two groups are less than a given threshold, are suitable (Engel, 2021, Lakens, 2017). In addition to post hoc power analyses and threshold comparative analyses, there is also a hypothesis comparative approach, the Bayesian Factors, to decide whether there is evidence for the null hypothesis or the alternative hypothesis and therefore to decide whether an effect is present or absent.

I compared MDD, EQIV, CI and BF for their reliability in detecting true negatives among non-significant results of a NHST, by comparing their rate to falsely mistrust a significant result, despite there is no effect (FMR) and their rate to falsely trust a non-significant result, despite there is an effect (FTR). My aim was to identify the best method for distinguishing between true negatives and false negatives to reliably establish no effect. Furthermore, I investigated the consequences that come along with the recent switch to the use of Equivalence tests instead of MDDs in risk assessment of European Food Safety Authority (EFSA).

## 2. Review of statistical approaches to establish no effect

### 2.1 NHST

*The hypotheses.* In science in general, and here with a focus on ecotoxicology, hypothesis testing is a statistical method used to determine whether observed differences are random or systematic (Schönbrodt, 2017). Systematic differences between two observed groups indicate that a variable, e.g. chemical exposure, has an effect on a particular parameter, e.g. the average survival rate of each two groups. One of the groups is the control group, which is not exposed to the chemical but is matched to the same conditions as the treatment group, which is exposed to the chemical. NHSTs are designed to test a previously formulated null hypothesis, which states that there is no difference or no relationship between distributions or parameters (Krueger, 2001). If differences in the parameters in both directions are of interest, a two-sided test is performed, and if differences in only one direction are of interest, a one-sided test is performed. When  $Parameter_{treatment}$  is the distribution of data from the group treated with chemicals and  $Parameter_{control}$  is the distribution of data from the control group, then the null hypothesis ( $H_0$ ) and alternative hypothesis ( $H_1$ ) are:

$$H_0: Parameter_{treatment} - Parameter_{control} = 0 \text{ (two - sided)}$$

$$H_1: Parameter_{treatment} - Parameter_{control} \neq 0$$

$$H_0: Parameter_{treatment} - Parameter_{control} \leq 0 \text{ (one - sided)}$$

$$H_1: Parameter_{treatment} - Parameter_{control} > 0$$

The alternative hypothesis  $H_1$  is complementary to the null hypothesis and refers to the same parameters as  $H_0$ . The null hypothesis is the basis for statistical inference concluded by the principle of falsification (Le, 2003).

*The significance level.* To falsify or verify the null hypothesis, the Null Hypothesis Significance Test uses a significance level  $\alpha$ , which is a predetermined threshold to determine the level of evidence required to falsify a null hypothesis (Gill, 1999). The significance level indicates a certain chance that the null hypothesis will be falsely rejected, resulting in a false positive also called Type I error (Banerjee, 2009).

*The error types.* In hypothesis testing, two types of error can occur: A Type I error and a Type II error. When a type I error occurs, the  $H_0$  is erroneously rejected, and when a type II error occurs, the  $H_0$  is erroneously not rejected, thus Type I and Type II error behave contrary (Akobeng, 2016). A reduction in Type I error would, under the same conditions, lead to a corresponding increase in Type II error. Both errors depend on the sample size: the larger the sample size, the lower the risk of either Type I error or Type II error occurring (Harmon, 2005). As mentioned above, type I error usually has a probability equal to the significance level  $\alpha$  and Type II error has a probability denoted by  $\beta$  (Pollard, 1987).

*The power.*  $(1 - \beta)$  is the so-called statistical power. A high statistical power indicates a high probability of rejecting the null hypothesis when it is false (Källén, 2011).

*The p-value.* In NHST the p-value is often used to decide whether to reject or not to reject  $H_0$ : If the p-value is smaller than or equal to the significance level  $\alpha$ , the  $H_0$  is rejected and if the p-value is larger than the significance level  $\alpha$ ,  $H_0$  is not rejected (Martínez-Abraín, 2008). Greenland (2016) explained that a p-value less than, greater than, or equal to  $\alpha$  only means that a deviation from the hypothesized prediction would be as large as or larger than the observed one in at most  $\alpha$  of the cases if the deviation were caused by chance alone. Therefore, the p-value can be defined as the probability that the values of the test statistic are as extreme as or more extreme than those observed when the null hypothesis is true (Le, 2003).

The NHST is a useful tool to assume whether the null hypothesis is true, but it fails to find out how far away the true effect is from zero if the null hypothesis was not rejected (Lakens, 2017). Therefore, one needs to decide whether to trust or to mistrust non-significant results (Mair, 2020). Trust or mistrust is determined by the Type I or Type II error rates of a statistical method.

## 2.2 Post-hoc power analysis

*The MDD.* The MDD is the minimum effect size, that would lead to significant results when comparing the means of two different groups in a set of experiments (Duquesne, 2020). It can be calculated as follows (Mair, 2020):

$$1) MDD = t_{critical} \times s \times \sqrt{\frac{1}{n_{control}} + \frac{1}{n_{treatment}}}$$

Where  $t_{critical}$  is the critical value of the test statistic,  $s$  is the residual standard deviation and  $n_{control}$  and  $n_{treatment}$  are the number of observations in each group. The MDD considers the power of an experiment and can therefore be used to determine an appropriate sample size (Brock, 2015). I calculated the MDD in R Studio using the above-mentioned function (1).

## 2.3 Threshold comparative analyses

*Equivalence Tests.* EFSA (2010) defined equivalence as the absence of differences in a certain parameter between a treated group and an untreated control group. The difference between these groups is regarded as ‘acceptable’ if it lays within the prespecified, limiting equivalence thresholds (Lakens, 2017). If  $\Delta$  ( $>0$ ) is the prespecified acceptable difference between two distributions (the limiting equivalence threshold), the null hypothesis for a one-sided equivalence t-Test and the alternative hypothesis are formulated as follows (Tango, 1998):

$$\begin{aligned} H_0: Parameter_{treatment} - \Delta - Parameter_{control} &\geq 0 \\ H_1: Parameter_{treatment} - \Delta - Parameter_{control} &< 0 \end{aligned}$$

If results are significant, then the effect is regarded as equivalent to zero and the corresponding chemical concentration is accepted. When testing for equivalence, either one or two one-sided tests can be performed, depending on whether one is interested in detecting only an increase or a decrease or both (EFSA, 2010). I was interested in finding out whether the treatment group has greater parameters than the control parameters or not. Therefore, I used the `t.test()` function in RStudio to test for equivalence in one direction.

*Confidence Intervals.* The CI is an interval estimate, which measures the accuracy of the estimation of a parameter by providing an interval within which the parameter is assumed to lie. The interval of values that includes the 'true' value is estimated by a given statistic with a given probability (Nakagawa, 2007). Most used CIs are the 90%, 95% and 99% CI (Simundic, 2008). In a 95% CI for example will the real effect be larger than the upper and smaller than the lower CI bound in 5 % of the experiments. The CI informs about the hypothetical values that could not be rejected in a set of samples and is therefore used to represent statistical uncertainty of parameter estimation (Smithson, 2003). I extracted upper CIs from the `t.test()` function in RStudio.

## 2.4 Hypothesis comparative analyses

*Bayesian Factor analysis.* The Bayesian approach is a probabilistic inference method used to adjust the estimates of a parameter (prior) based on observed data, resulting in a new probability distribution (posterior) for the parameter of interest (Eddy, 2004). The posterior probability distribution  $P(H|D)$  can be calculated using the Bayes-Theorem (Bolstad, 2016 and Stöcklin): When  $P(D|H)$  is the likelihood, that gives the probability of the data under the assumption that the hypothesis is not rejected and  $P(H_1)$  is the prior, which is the assumption for the probability that the hypothesis is not rejected, then:

$$2) \text{ for } H_0: P(H_0|D) = \frac{P(D|H_0) \cdot P(H_0)}{P(D)}$$

$$\text{and } 3) \text{ for } H_1: P(H_1|D) = \frac{P(D|H_1) \cdot P(H_1)}{P(D)}$$

The Bayes factor compares the two hypotheses and decides then whether the result is evidence for the null hypothesis or the alternative hypothesis. The Bayes-Factor (BF) is the factor by which the prior is changed to the posterior and can be derived as follows:

$$4) \frac{P(H_1|D)}{P(H_0|D)} = \frac{P(D|H_1)}{P(D|H_0)} \cdot \frac{P(H_1)}{P(H_0)} = BF \cdot \frac{P(H_1)}{P(H_0)}$$

I computed the Bayes factors using the 'BayesFactor' package (from Richard D. Morey and Jeffrey N. Rouder) in RStudio.

## 3. Comparison of statistical Methods

My aim was to find out which statistical method is most appropriate to distinguish between true negative and false negative tests and how sample size affects the performance of MDD and equivalence tests. To solve these two questions, I generated two different simulations: the False Mistrust Rate/False Trust Rate (FMR/FTR)-simulation and the sample-size-simulation. Both simulations imply various statistical methods (like BF, CI, EQUIV, MDD) to assess the trustworthiness of non-significant ( $p\text{-value} > 0.05$ ) results derived from a one-sided t-test.

Results of the FMR/FTR-simulation were used to calculate Type I and Type II error rates. Since the error rates and the error control of the above-mentioned statistical methods are different. A Pareto plot (Fig. 2) gives a visual assessment of how well a statistical method discriminates between false positives and false negatives.

The sample-size-simulation implies EQUIV and MDD to analyze data sets with different sample sizes. Considering that European Food Safety Authority (EFSA) has switched from MDD to the use of equivalence tests in their risk assessment, I was interested in finding out how the acceptance of effects is affected by the experimental sample size.

### 3.1 Simulation

I generated data sets displaying a control and treatment group having the same standard deviation, the same length and being normal distributed. The only difference between the two groups lays in their means: in half of the datasets the treatment group has a higher mean than the control group, indicating a larger true effect size of the treatment group compared to the control group, while in the other half the mean is the same (see Fig. 2: 'Data set'). The generated data sets have either the same sample size (25) or have differing sample sizes (4:100, step size = 4).

I analyzed the data sets with simulations, that imply different statistical methods (BF, CI, EQUIV, MDD) to test whether to 'trust' or to 'mistrust' non-significant ( $p\text{-value} > 0.05$ ) results from a one-sided t-test (see Fig. 2: 'Simulation'). If the t-test p-value is greater than 0.05, the 'effect detected' variable is set to 'false', and if the result of the appropriate statistical methods meets the appropriate assumptions (Bayes factor  $\leq$  threshold, upper confidence interval  $\leq$  threshold, equivalence test p-value  $\leq 0.05$ , MDD  $\leq$  threshold), then the 'no effect trusted' variable is set to 'true' and the effect can be considered 'acceptable low'. Accepting an effect depends on how high the threshold is set. A threshold must be chosen to translate the MDD, the CI, the EQUIV and the BF into a trust/mistrust decision. This allows the error rates of this decision (FTR and FMR) to be calculated for the different methods and thresholds. A set of thresholds (0:1.1, step size = 0.1) or a pre-calculated threshold is implemented in the respective FMR/FTR-simulation (Fig. 1: marked with purple color) and sample-size-simulation (Fig. 1: marked with blue color). For the sample-size-simulation, I calculated the threshold as in the statistical considerations of European Food Safety Authority (EFSA, 2010) (equations 5 and 6). Where 'lsd' stands for the least significant difference (equivalent to MDD), 'df' for degrees of freedom, 'i' for the model, 'a' for the significance level, 'XY' for the two groups and 'sed' for the standard error.

$$5) \text{ threshold} = \exp(\text{mean}_{\text{treatment}} - \text{mean}_{\text{control}} - \text{lsd})$$

$$6) \text{ lsd} = t(df; i; a) * \text{sed}(XY; i)$$

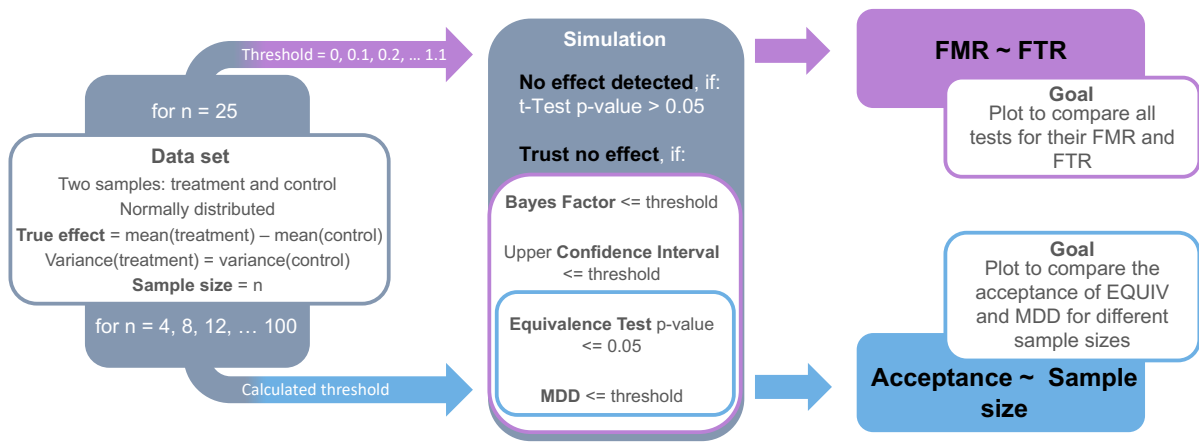
I used the results of the FMR/FTR-simulation to calculate the False Trust Rate (FTR) and the False Mistrust Rate (FMR) for each statistical method. The calculation of the two rates is based on the calculations of Mair et al. (2020) (equations 7 and 8). I plotted the FMR against the FTR (Section 4, Fig. 2) for visual comparison.

$$7) \text{ FTR} = \text{Trust} \left[ \frac{\sum(\text{no effect detected} \ \& \ (\text{true effect} > 0))}{\sum(\text{true effect} > 0)} \right]$$



$$8) FMR = 1 - \text{Trust} \left[ \frac{\sum(\text{no effect detected} \ \&(\text{true effect} = 0))}{\sum(\text{true effect} = 0)} \right]$$

To examine the dependence of acceptance on sample size, I calculated the average 'no effect trusted' for each dataset of a certain length from the results of the sample-size-simulation. The result variable 'no effect trusted' is dichotomous, and thus '1' stands for accepted and '0' for not accepted. I plotted the average 'no effect trusted' variable against the length of the corresponding data set (Section 4, Fig. 3) for visual comparison.



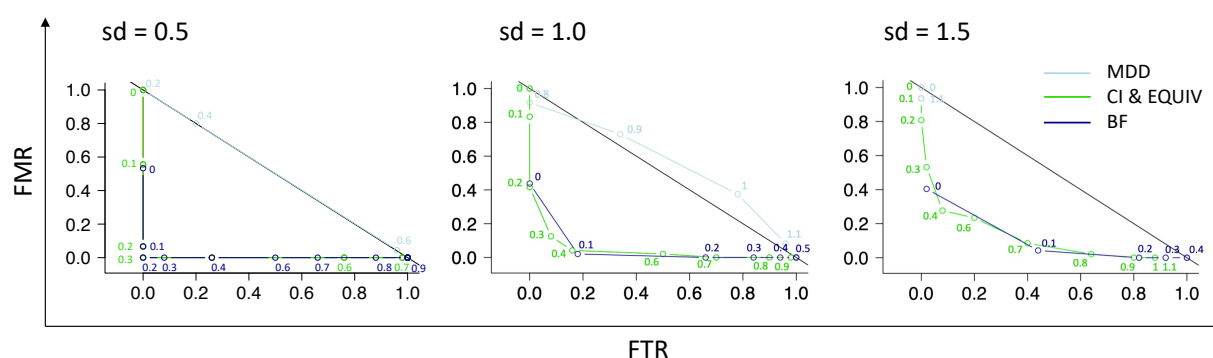
**Figure 1: Schematic illustration of the R code workflow.** The data sets display control and treatment groups with matching standard deviations, lengths, and normal distributions. Half of the data sets have the treatment group with a higher mean, indicating a larger true effect, while the other half have the same mean compared to the control group. The sample sizes of the generated data sets are either identical ( $n = 25$ ) or different ( $n = 4:100$ , step size = 4) for FMR/FTR-simulation (purple) and sample-size-simulation (blue) respectively. Different thresholds are used in the two simulations. Both simulations use various statistical methods (BF, CI, EQUIV, MDD) to assess the trustworthiness of non-significant ( $p\text{-value} > 0.05$ ) results from an one-sided t-test. If the t-test  $p\text{-value}$  exceeds 0.05, the 'effect detected' variable is set to 'false', and if the results of the relevant statistical methods meet certain assumptions (Bayes factor  $\leq$  threshold, upper confidence interval  $\leq$  threshold, equivalence test  $p\text{-value} \leq 0.05$ ,  $MDD \leq$  threshold), then the 'no effect trusted' variable is set to 'true'. The results of each simulation are plotted, and the resulting plots provide a visual comparison of the statistical methods.

## 4. Results and Discussion

### What is the most appropriate method for distinguishing true negatives from false negatives?

When comparing the performance of the four statistical methods, I found that CI (Fig. 3, green line) consistently performed exactly like EQUIV (Fig. 3, green line), regardless of the threshold or variance chosen. With increasing variance, the ability to discriminate between false trust and false mistrust of non-significant results of CI, EQUIV and BF (Fig 3, dark blue line) decreased. This decrease is because statistical power decreases as the variance of the data increases (McClelland, 2000). A reduction in power is associated with higher Type II error,

leading to higher FMR and FTR (see Section 2, 'Power'). The patterns of BF compared to CI & EQUIV were almost indistinguishable, especially at a standard deviation of 0.5 (see Fig. 2). The difference between them is the need to use different thresholds for CI, EQUIV and BF to achieve equivalent false trust and false mistrust rates. BF, CI & EQUIV outperformed MDD in all cases, showing much lower FTR and FMR than MDD. MDD is therefore less powerful for detecting true negatives among nonsignificant results than BF, CI & EQUIV. The reason for this might be that MDD is defined independently of the estimated effect size (Mair, 2021). Otherwise MDD appears to behave approximately random (see Appendix, Fig.4: Random), which would imply that MDD cannot systematically discriminate between trust and mistrust, meaning that MDD does not help to show any tendency in which one would falsely trust or mistrust non-significant results. Therefore, the use of MDD in general would be questionable. My findings on the performance of MDD contradict those of Mair et al. (2020): They found that MDD determines whether a non-significant result indicates a true lack of effect. This contradiction could be due to something going wrong with my simulation of the MDD. To find the error in my simulation, I tested the use of different variances, thresholds, and effect sizes; the behavior of the MDD curve in the Pareto plot did not change. In addition, instead of calculating the MDD (see Section 2, MDD), I used the MDD function from Mair et al. (2020) but the behavior did not change here either.



**Figure 2: Application of different thresholds to see the tendency of statistical methods to falsely trust or mistrust non-significant results.** A threshold (circle with corresponding value) must be chosen to translate the Minimum Detectable Difference (MDD), the Confidence Interval (CI), the Equivalence Test (EQUIV) and the Bayes Factor (BF) into a trust/mistrust decision. This decision allows error rates (False Trust Rate (FTR) and False Mistrust Rate (FMR)) to be calculated for the different methods and thresholds. Each of the three plots shows the FTR/FMR results for each statistical method on data sets with different standard deviations. With increasing standard deviation, the ability of CI (green line), EQUIV (green line) and BF (dark blue line) to discriminate between trust and mistrust of non-significant results decreased. CI consistently performed the same as EQUIV, regardless of the threshold or variance chosen (both are on the same line, with the same thresholds). BF, CI & EQUIV outperformed MDD in all cases, showing lower FTR and FMR compared to MDD (light blue line), which behaved in all scenarios approximately random. The patterns of BF compared to CI & EQUIV were almost indistinguishable, especially at a standard deviation of 0.5. Here, the only difference was the need to use different thresholds for CI & EQUIV and BF to achieve equivalent FMR and FTR.

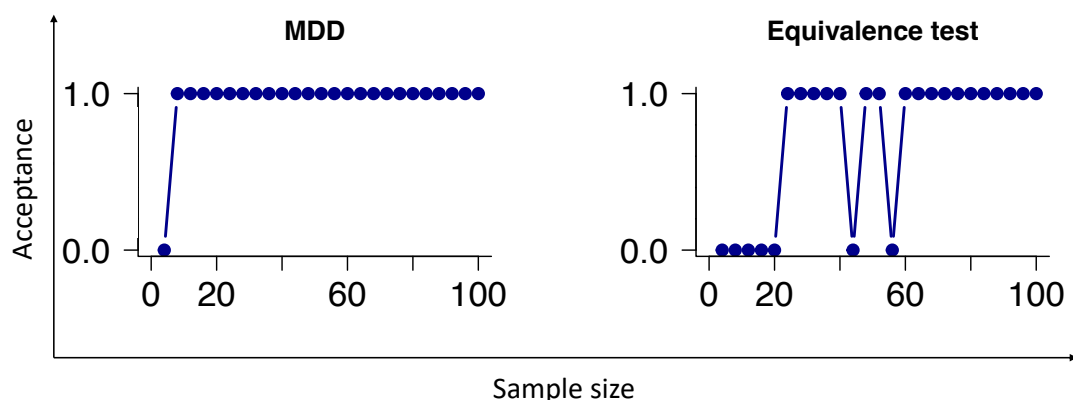
## Old EFSA guidelines compared to new EFSA guidelines

The goal of ecotoxicological experiments is to derive a NOEC concentration or a no-observed ecologically adverse effect concentration (NOEAE). NOECs from ecotoxicological tests that

are statistically significant ( $p\text{-value} \leq 0.05$ ) should be evaluated. The evaluation of NOEC is possible if the statistical power is high enough ensured by an appropriate number of species/taxa concentration–response relationships. For post hoc evaluation of statistical power, EFSA has considered MDD to be a valid concept. The calculation of the MDD allows the reporting of the actual effect which could be determined in the experiment for a given endpoint. MDD was used in European Food Safety Authority (EFSA) risk assessment in the last decade (Europäische Kommission, 2013, EFSA, 2016, EFSA 2017, EFSA, 2019). Now European Food Safety Authority (EFSA) switched to using Equivalence Tests in its ecotoxicological risk assessment: In the current risk assessment of plant protection products on bees, the derivation of a single effect estimate (NOEC) is no longer relevant, as the risk assessment is based on dose–response relationships with the goal to consider the predicted level of effect triggered by different exposure levels. For estimating the levels of risk Equivalence tests are conducted to statistically analyze higher tier semi-field or field studies with the goal to identify relevant hazard parameters or ‘effect endpoints’. Effect endpoints are the combination of the chosen dose-response model and the values of its parameters and give a link between exposure in the field and effects in an experiment. European Food Safety Authority (EFSA) recommends to apply a one-sided equivalence test ( $\alpha = 0.2$ ) for each endpoint, with an equivalence limit corresponding to a 10% reduction in the treated test compared to the control, to prove that there are no adverse effects (EFSA, 2023).

## **Can the Equivalence Test compete with MDD for small sample sizes?**

The change from the use of MDD to the use of Equivalence tests in EFSA risk assessment raises questions of applicability. In practice, statistical methods use the data from animal experiments in which the number of animals tested, is to be kept as small as possible. My sample size simulation (section 3) shows how experimental sample size affects the acceptance of effects by the two different methods. To simulate the equivalence test, I used the threshold (equation 5) derived from EFSA's 2010 statistical considerations. Figure 3 plots the decision to accept or reject no effect (y-axis, 0.0 = not accepted and 1.0 = accepted) against sample size. The MDD accepts no-effect concentrations from a sample size of 8 on, whereas the Equivalence test starts to accept no-effect concentrations from a sample size of 24 and becomes robust from a sample size of 60. The graphs show that the new EFSA guidelines, which require the use of equivalence tests, result in a higher number of animals tested to establish no-effect concentrations. Compared to other statistical tests MDD has been shown to have relatively high power. The power of MDD is even increasing with decreasing sample size (Mair, 2020). This is an important feature, because ecotoxicological risk assessment aims to minimise the number of higher tier experiments.



**Figure 3: Acceptance of no-effect concentrations in different experimental sample sizes.** The y-axis displays the decision to accept or to reject no-effect: 0.0 == not accepted and 1.0 == accepted. The Minimum Detectable Difference (MDD) accepts no-effect concentrations from a sample size of 8 on, whereas the Equivalence test starts to accept no-effect concentrations from a sample size of 24 and becomes robust from a sample size of 60 on.

## 5. Summary and conclusion

In this study I wanted to find out which statistical method is most appropriate to distinguish between true negative and false negative tests and how sample size affects the performance of MDD and equivalence tests in accepting no effect concentrations. My main results are that Confidence Intervals and Equivalence tests are performing the same in identifying true effects if they are present and that the new European Food Safety Authority (EFSA) guidelines with the use of Equivalence tests require more animal testing than in the past. In conclusion, I support the findings of Mair et al. (2020) in recommending the use of CIs for the interpretation of non-significant results. The reduced error rates of CIs (and Equivalence tests) ensure reliable results and unlike equivalence tests, CIs are a common method that most scientists have in their statistical toolbox.

## Bibliography

- Fay, M. P., Halloran, M. E., & Follmann, D. A. (2007). Accounting for variability in sample size estimation with applications to nonadherence and estimation of variance and effect size. *Biometrics*, 63(2), 465–474.
- Aguilar-Marcelino, L., Al-Ani, L. K. T., Wong-Villarreal, A., & Sotelo-Leyva, C. (2023). Persistence of pesticides residues with chemical food preservatives in fruits

- and vegetables. In *Current Developments in Biotechnology and Bioengineering* (pp. 99–118). Elsevier.
- Akobeng, A. K. (2016). Understanding type I and type II errors, statistical power and sample size. *Acta Paediatrica*, 105(6), 605–609.
- Banerjee, A., Chitnis, U. B., Jadhav, S. L., Bhawalkar, J. S., & Chaudhury, S. (2009). Hypothesis testing, type I and type II errors. *Industrial Psychiatry Journal*, 18(2), 127.
- Bolstad, W. M., & Curran, J. M. (2016). *Introduction to Bayesian statistics*. John Wiley & Sons.
- Brock, T. C. M., Hammers-Wirtz, M., Hommen, U., Preuss, T. G., Ratte, H. T., Roessink, I., Strauss, T., & Van den Brink, P. J. (2015). The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environmental Science and Pollution Research*, 22, 1160–1174.
- Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, 6(2), 48–60.
- Committee, E. S. (2016). Recovery in environmental risk assessments at EFSA. *EFSA Journal*, 14(2), 4313.
- Dienes, Z. (2014). Using Bayes to get the most out of non-significant results. *Frontiers in Psychology*, 5, 781.
- Duquesne, S., Alalouni, U., Gräff, T., Frische, T., Pieper, S., Egerer, S., Gergs, R., & Wogram, J. (2020). Better define beta-optimizing MDD (minimum detectable difference) when interpreting treatment-related effects of pesticides in semi-field and field studies. *Environmental Science and Pollution Research*, 27, 8814–8821.

- Eddy, S. R. (2004). What is Bayesian statistics? *Nature Biotechnology*, 22(9), 1177–1178.
- EFSA. (2010). Scientific opinion on statistical considerations for the safety evaluation of GMOs. *EFSA Journal*, 8(1), 1250–1311.
- EFSA, E. P. on G. M. (2010). Statistical considerations for the safety evaluation of GMOs. *EFSA Journal*, 8(2), 1250.
- EFSA. (2016). *Peer review of the pesticide risk assessment of the active substance flurtamone*. <https://doi.org/10.2903/j.efsa.2016.4498>
- EFSA. (2017). *Updated peer review of the pesticide risk assessment of the active substance flurtamone*. <https://doi.org/10.2903/j.efsa.2017.4976>
- EFSA. (2019). *Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology*. <https://doi.org/10.2903/sp.efsa.2019.EN-1673>
- EFSA, E. F. S., Adriaanse, P., Arce, A., Focks, A., Ingels, B., Jölli, D., Lambin, S., Rundlöf, M., Süßenbach, D., & Del Aguila, M. (2023). Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. And solitary bees). *EFSA Journal*, 21(5), e07989.
- Engel, J., & van der Voet, H. (2021). Equivalence tests for safety assessment of genetically modified crops using plant composition data. *Food and Chemical Toxicology*, 156, 112517.
- Europäische Kommission. (2013). *Verordnung (EU) Nr. 284/2013 der Kommission vom 1. März 2013 zur Festlegung der Datenanforderungen für Pflanzenschutzmittel gemäß der Verordnung (EG) Nr. 1107/2009 des Europäischen Parlaments und des Rates über das Inverkehrbringen von Pflanzenschutzmitteln*.

- Faller, H. (2004). Signifikanz, Effektstärke und Konfidenzintervall. *Die Rehabilitation*, 43(03), 174–178.
- Fenik, J., Tankiewicz, M., & Biziuk, M. (2011). Properties and determination of pesticides in fruits and vegetables. *TrAC Trends in Analytical Chemistry*, 30(6), 814–826.
- Gill, J. (1999). The insignificance of null hypothesis significance testing. *Political Research Quarterly*, 52(3), 647–674.
- Greenland, S., Senn, S. J., Rothman, K. J., Carlin, J. B., Poole, C., Goodman, S. N., & Altman, D. G. (2016). Statistical tests, P values, confidence intervals, and power: A guide to misinterpretations. *European Journal of Epidemiology*, 31, 337–350.
- Harmon, L. J., & Losos, J. B. (2005). The effect of intraspecific sample size on type I and type II error rates in comparative studies. *Evolution*, 59(12), 2705–2710.
- Heaton, C. A. (1993). *The chemical industry*. Springer Science & Business Media.
- Hickey, G. (2010). *Ecotoxicological risk assessment: Developments in PNEC estimation*. Durham University.
- Hoekstra, J. A., & Van Ewijk, P. H. (1993). Alternatives for the no-observed-effect level. *Environmental Toxicology and Chemistry: An International Journal*, 12(1), 187–194.
- McClelland, G. H. (2000). *Increasing statistical power without increasing sample size*.
- Isenring, R. (2010). Pesticides and the loss of biodiversity. *Pesticide Action Network Europe, London*, 26.
- Källén, A. (2011). *Understanding biostatistics*, 5-13. John Wiley & Sons.
- Kaur, P., & Stoltzfus, J. (2017). Type I, II, and III statistical errors: A brief overview. *International Journal of Academic Medicine*, 3(2), 268–270.

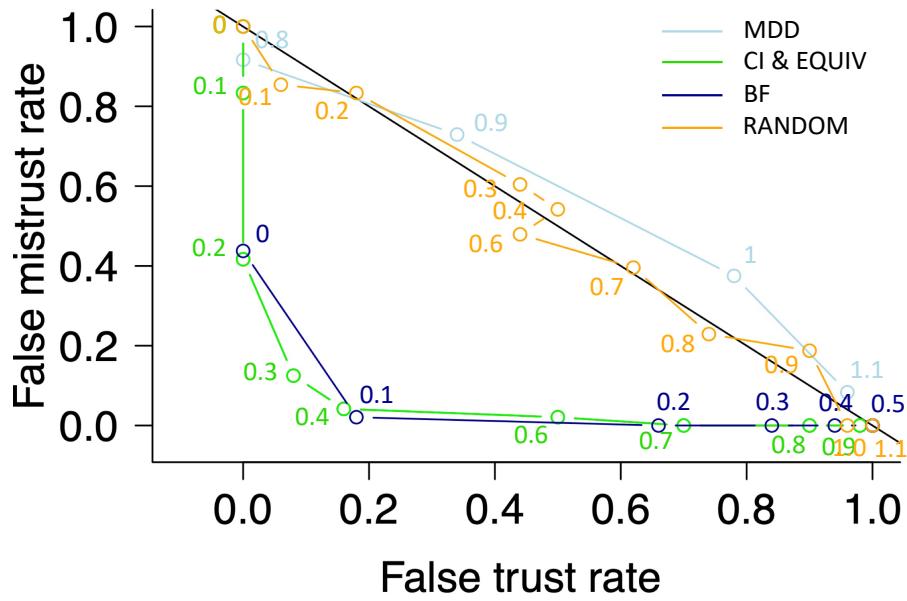
- Kendall, R. J., Anderson, T. A., Baker, R. J., Bens, C. M., Carr, J. A., Chiodo, L. A., Cobb III, G. P., Dickerson, R. L., Dixon, K. R., & Frame, L. T. (2001). *Ecotoxicology. USDA National Wildlife Research Center-Staff Publications*, 516.
- Krueger, J. (2001). Null hypothesis significance testing: On the survival of a flawed method. *American Psychologist*, 56(1), 16.
- Lakens, D. (2017). Equivalence tests: A practical primer for t tests, correlations, and meta-analyses. *Social Psychological and Personality Science*, 8(4), 355–362.
- Le, C. T. (2003). *Introductory biostatistics*, 188-208. John Wiley & Sons.
- Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). Effects of pesticides on environment. *Plant, Soil and Microbes: Volume 1: Implications in Crop Science*, 253–269.
- Mair, M. M., Kattwinkel, M., Jakoby, O., & Hartig, F. (2020). The minimum detectable difference (MDD) concept for establishing trust in nonsignificant results: A critical review. *Environmental Toxicology and Chemistry*, 39(11), 2109–2123.
- Martínez-Abraín, A. (2008). Statistical significance and biological relevance: A call for a more cautious interpretation of results in ecology. *Acta Oecologica*, 34(1), 9–11.
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews*, 82(4), 591–605.
- Nesheim, O. N., & Criswell, J. T. (1978). *Toxicity of pesticides*. Oklahoma State University, Cooperative Extension Service.



- Newman, M. C. (2008). "What exactly are you inferring?" A closer look at hypothesis testing. *Environmental Toxicology and Chemistry: An International Journal*, 27(5), 1013–1019.
- Oltmanns, J., Bohlen, M.-L., Escher, S., Schwarz, M., & Licht, O. (2019). Applying a tested procedure for the identification of potential emerging chemical risks in the food chain to the substances registered under REACH-REACH 2: External scientific report. OC/EFSA/SCER/2016/01-CT 1. *EFSA Supporting Publications*, 16(3), 1597E.
- Pollard, P., & Richardson, J. T. (1987). On the probability of making Type I errors. *Psychological Bulletin*, 102(1), 159.
- Satterthwaite, F. E. (1941). Synthesis of variance. *Psychometrika*, 6(5), 309–316.
- Schönbrodt, F. D., Wagenmakers, E.-J., Zehetleitner, M., & Perugini, M. (2017). Sequential hypothesis testing with Bayes factors: Efficiently testing mean differences. *Psychological Methods*, 22(2), 322.
- Simundic, A.-M. (2008). Confidence interval. *Biochemia Medica*, 18(2), 154–161.
- Skalski, J. R. (1981). *Statistical inconsistencies in the use of no-observed-effect levels in toxicity testing*. ASTM International.
- Smithson, M. (2003). *Confidence intervals* (Issue 140). Sage.
- Srivastava, M. K., & Raizada, R. B. (1999). Assessment of the no-observed-effect level (NOEL) of quinalphos in pregnant rats. *Food and Chemical Toxicology*, 37(6), 649–653.
- Stöcklin, M. (n.d.). *Einführung in die Bayessche Statistik*.  
<https://mmi.psychologie.unibas.ch/r-toolbox/Skripte/Bayes%20Einfuehrung.pdf>

- Swindlehurst, R. J., Johnston, P. A., Tröndle, S., Stringer, R. L., Stephenson, A. D., & Stone, I. M. (1995). Regulation of toxic chemicals in the Mediterranean: The need for an adequate strategy. *Science of the Total Environment*, 171(1–3), 243–264.
- Tango, T. (1998). Equivalence test and confidence interval for the difference in proportions for the paired-sample design. *Statistics in Medicine*, 17(8), 891–908.
- van der Werf, H. M. (1996). Assessing the impact of pesticides on the environment. *Agriculture, Ecosystems & Environment*, 60(2–3), 81–96.
- Wang, G. M. (1988). Regulatory decision making and the need for and the use of exposure data on pesticides determined to be teratogenic in test animals. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 8(2), 117–126.
- Ware, G. W. (1980). Effects of pesticides on nontarget organisms. *Residue Reviews: Residues of Pesticides and Other Contaminants in the Total Environment*, 173–201.
- Warne, M. S. J., & Van Dam, R. (2008). NOEC and LOEC data should no longer be generated or used. *Australasian Journal of Ecotoxicology*, 14(1), 1–5.
- Williams, C. M. (1967). Third-generation pesticides. *Scientific American*, 217(1), 13–17.
- Williams, E. S., Berninger, J. P., & Brooks, B. W. (2011). Application of chemical toxicity distributions to ecotoxicology data requirements under REACH. *Environmental Toxicology and Chemistry*, 30(8), 1943–1954.

## Appendix



**Figure 4: Application of different thresholds to see the tendency of statistical methods to falsely trust or mistrust non-significant results.** A threshold (circle with corresponding value) must be chosen to translate random values (Random), the Minimum Detectable Difference (MDD), the Confidence Interval (CI), the Equivalence Test (EQUIV) and the Bayes Factor (BF) into a trust/mistrust decision. This decision allows error rates (False Trust Rate (FTR) and False Mistrust Rate (FMR)) to be calculated for the different methods and thresholds. Each of the three plots shows the FTR/FMR results for each statistical method on data sets with different standard deviations, representing the variation in the samples. With increasing standard deviation, the ability of CI (green line), EQUIV (green line) and BF (dark blue line) to discriminate between trust and mistrust of non-significant results decreases. MDD (light blue line) behaves in all scenarios approximately random (orange line), indicating that MDD does not help to show any tendency in which one would falsely trust or mistrust non-significant results. CI consistently performed the same as EQUIV, regardless of the threshold or variance chosen (both are exactly on the same line, with the same thresholds). The patterns of BF compared to CI & EQUIV were almost indistinguishable, especially at a standard deviation of 0.5 (see Fig. 2). Here, the only difference was the need to use different thresholds for CI & EQUIV and BF to achieve equivalent false trust and false mistrust rates