
# Pain Relief Medication

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## Executive Summary

Based on an experiment evaluating pain response following the administration of marijuana and morphine in mice, this report details suggestive evidence of a synergistic interaction between the two drugs and identifies their minimal effective dosages. With data provided to Diché Consulting, analytical findings reveal a minimal effective dosage of 4.0 mg/kg for marijuana and 5 mg/kg for morphine. Potential for drug synergy was also found at a dose of 1.5 mg/kg for both drugs. However, the estimated degree of synergy may be overstated due to inadequate choices for marijuana doses. Diché Consulting proposes a strategy to supplement current experimental data to enhance accuracy of results and identify minimal dosages necessary for optimal efficacy.

## Introduction

Diché Consulting has been appointed by a pharmaceutical enterprise, henceforth referred to as "the Company", to determine the minimum dosage amount of a drug that is part of their medication development process. The Company has undertaken an experiment consisting of 20 trials to assess the effects of marijuana and morphine, both independently and in combination.

The trials were divided into:

- 1 control trial;
- 5 trials using marijuana only;
- 8 trials using morphine only;
- 6 trials using both drugs.

In each of the experimental trials, except for the control, a specific dosage of one or both drugs were administered to 10 mice. After an appropriate duration to allow the drug(s) to take effect, the mice are subjected to a tail flick test. This type of test involves applying a pain stimulus to the tail of a mouse and observing whether the mouse flicks its tail or not. The absence of tail flicking indicates efficacy of the drug for that mouse, whereas tail flicking implies no significant effect. The number of mice that do not exhibit tail flicking is tallied for each trial. This count is used to calculate the proportion of the 10 mice who do not flick their tail. This proportion is regarded as the average drug effect for the chosen dosage of the trial. A higher proportion signifies a greater effect and this connection between magnitude of effect and dosage is commonly referred to as a **dose-response relationship**.

## Objectives

The separate use of marijuana and morphine as analgesics has been well-established. Given the propensity for drugs with similar effects to be administered in combination, it is imperative to investigate potential advantages and effects of their co-administration.

The experiment has two objectives:

1. Determine minimal effective dosages for marijuana and morphine.
2. Determine if there is synergy between marijuana and morphine.

To provide clarity and precision, Diché Consulting has been enlisted by the Company to achieve these two objectives utilizing the data from the experiment. To meet these goals, it is essential to provide additional context and introduce relevant terminology.

## Contextualization

According to the Company's directives, a dosage is deemed **effective** if the corresponding effect is 50% or greater. It is also referred to as the **efficacy** of the dosage. Consequently, the minimal effective dosage for both drugs independently and in combination can be determined by identifying the trials where the dosages have the smallest quantities, yet yield a proportion of mice not flicking their tail at least 50% of the time.

To better understand the second objective pertaining to synergy, the isobole method is introduced.

When evaluating the concentration interactions of drugs, several statistical determinations are utilized, with the **isobole method** being one of them. This method is characterized by its curve, which is represented by either a straight line or occasionally by a non-linear function. In addition, the isobole relies on an assumption to have a linear shape. The two drugs under consideration must possess a **constant potency ratio**. The potency ratio is the ratio of dosages of the two drugs at the same effect level. For example, if 4 mg/kg of marijuana or 5 mg/kg of morphine (separately) are needed to obtain an effect level of 50%, then the potency ratio for the 50% effect level between marijuana and morphine is 4/5. If the potency ratio between marijuana and morphine remains identical for any effect level, it is called a constant potency ratio. This assumption allows the derivation of a linear isobole. The

isobole method facilitates the identification of whether those dosages are synergistic or antagonistic, but also points out the optimal combinations of dosages. A detailed methodology for creating the isobole will be presented in the following section. Once the isobole is constructed, it becomes intuitive and straightforward to understand how to define synergy, its presence, and to comment on the analysis results as the isobole construction proceeds.

## Isobole construction and results

To construct an isobologram, an initial step entails plotting the morphine dosages against marijuana dosages. With 20 trials conducted, each utilizing a different combination of dosages, the consequent graph should consist of 20 distinct points. Furthermore, as the effect of drugs on mice was determined for each trial by computing the proportion of mice that refrained from flicking their tail, these points can be color-coded. If the effect fails to meet the Company's prescribed threshold of 50%, the corresponding point will be designated with a teal hue. This indicates that the dosages of that trial are deemed ineffective. Conversely, points colored in red are effective and exceed the required threshold.

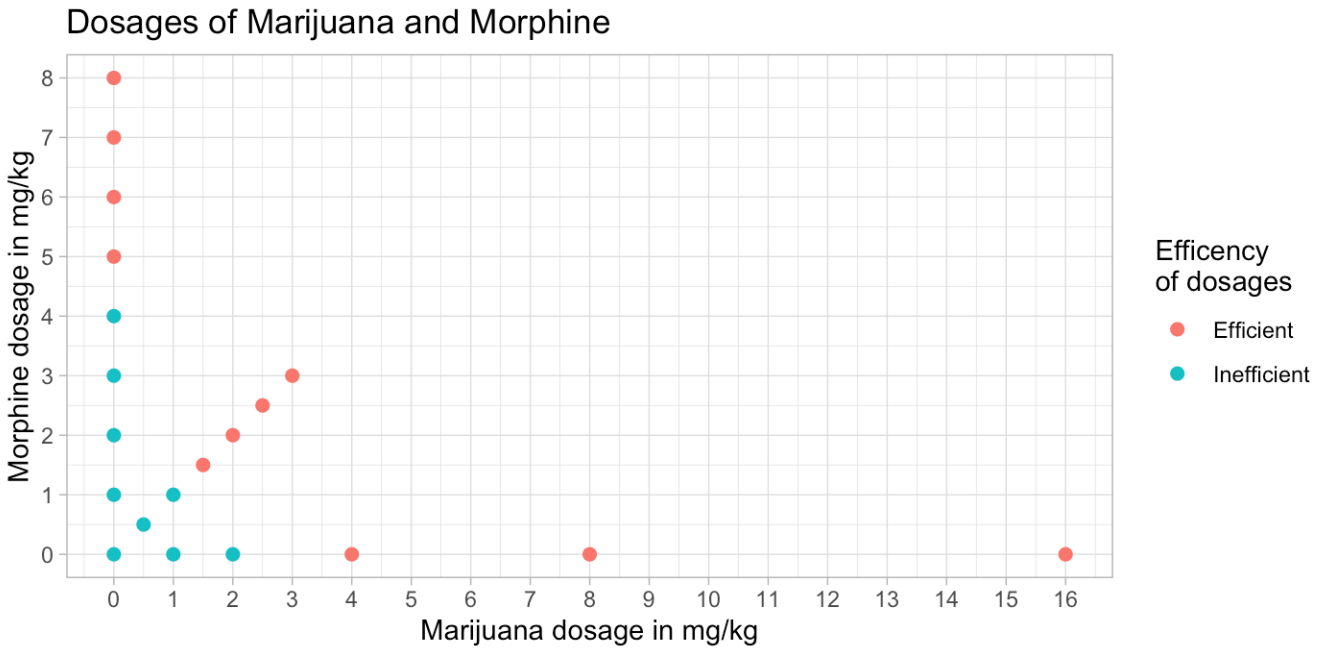


***Figure 1***: Dosages of Marijuana and Morphine with color coding according to the efficacy of the dosages.

The first figure presents the dosages of marijuana and morphine used in the experiment. The color-coding allows us to observe that, for trials only using marijuana (points laying on the horizontal axis), dosages of 1 and 2 mg/kg are ineffective when compared to dosages of 4, 8 and 16 mg/kg. In contrast, trials using only morphine indicate that only the dosages from 5 to 8 mg/kg are effective, as depicted on the vertical axis. Notably, the origin of the graph represents the control trial, where most mice are subjected to the pain stimulus without any pain-alleviating drugs flicked their tail. The graph also enables us to quickly identify which kind of drug combinations were tested, as those points not lying on either axis follow a perfect diagonal line. This shows that only equal dosages of both drugs were administered. There are no trials including both drugs where the amount of marijuana given to mice is greater or less than the amount of morphine.

Furthermore, the minimal effective dosages can be inferred from the first graph that we can already know. All effective dosages are represented in red, and the ones that are minimal in quantity are the closest to the origin. We can conclude that:

- 4.0 mg/kg is the minimal effective dosage for marijuana alone;
- 5.0 mg/kg is the minimal effective dosage for morphine alone;
- 1.5 mg/kg of marijuana and 1.5 mg/kg of morphine is the minimal effective dosage for both drugs.

To draw the isobole, we must first determine the two points at which morphine and marijuana, used independently, reach an effect level of 50%. To accomplish this, we display the dose-response curve for both drugs.

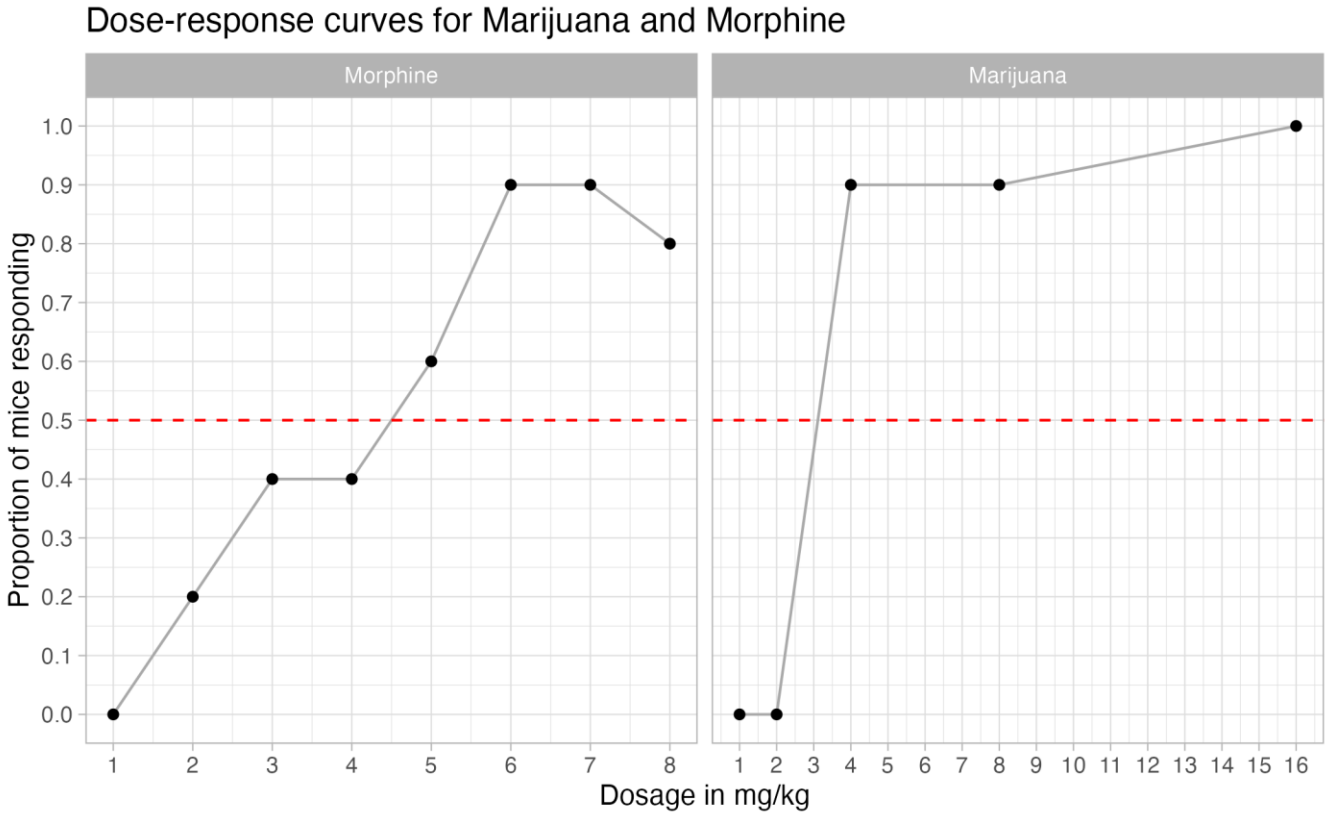


***Figure 2**:* Dose-response curves for each drug with 50% effect level highlighted by a red line.

It is evident that none of the dosages tested achieved exactly an effect level of 50%. However, there are notable differences between the effects of morphine and marijuana. Specifically, the morphine dosages exhibit a progressive increase in drug efficacy, whereas marijuana dosages transition from a null effect to a significant majority of mice responding to the drug. This does not give a precise idea of how the effect evolves between 2 mg/kg and 4 mg/kg of marijuana. In the subsequent section, we will expand more on this issue after having constructed the isobole. At present, based on the shape of the dose-response curves, Diché have made the following **assumptions**:

- 4.5 mg/kg is the theoretical dosage achieving a 50% effect level for morphine;
- 3.0 mg/kg is the theoretical dosage achieving a 50% effect level for marijuana.

We incorporate these two dosages into our prior dosage plot and draw the isobole which is characterized as the line connecting the two points together.

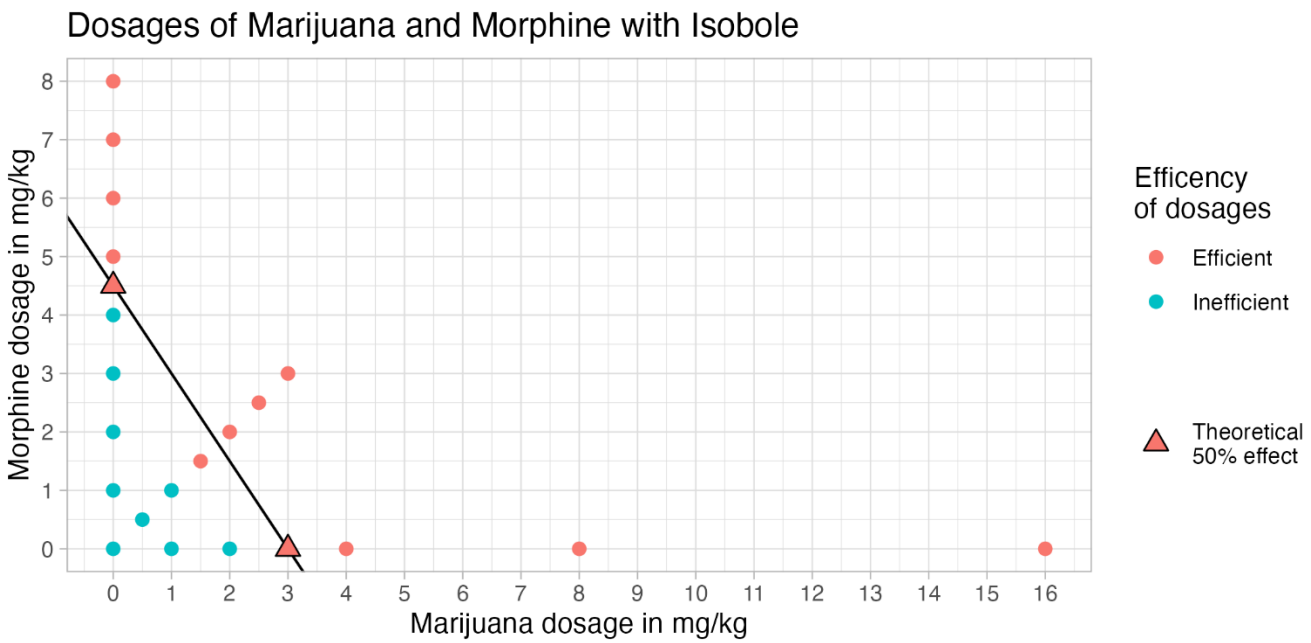


***Figure 3**:* Dosages of Marijuana and Morphine with theoretical 50% effect dosages and isobole.

After constructing the isobole, we proceed to explain its interpretation. The two triangles we added into the graph represent the theoretical dosages that achieve a 50% level effect for marijuana and morphine separately. Accordingly, it can be inferred that using 4.5 mg/kg of morphine is equivalent to using 3.0 mg/kg of marijuana. **Assuming that morphine and marijuana act independently**, that is, they do not interact even when used together, then:

- Every point situated **on** the isobole represents a combination of dosages which gives a 50% effect level (for example, using 1 mg/kg of marijuana and 3 mg/kg of morphine is equivalent to using 4.5 mg/kg of morphine or using 3 mg/kg of marijuana, they should all have a 50% effect level);
- Every point situated **above** the isobole represents a combination of dosages which gives an effect level greater than 50% (represented by a red point);
- Every point situated **under** the isobole represents a combination of dosages which gives an effect level of less than 50% (represented by a teal point).

As the experimental results are available, we can quickly verify the hypothesis above. As seen in the graph, we observe that the dosage point corresponds to 1.5 mg/kg of each drug is situated under the isobole, yet it is colored red. This signifies that marijuana and morphine do not always act independently, there appears to be interaction between them for at least one dosage combination. Despite being below the isobole, this dosage attains an effect level of at least 50%. Thus, it is called a **synergistic** dosage, and we say that there is **synergy** between the two drugs at that specific dosage.

## Robustness of Assumptions

In the preceding section, Diché Consulting deployed the isobole method, which is based on two crucial assumptions:

1. Constant potency ratio assumption;
2. Theoretical dosages achieving 50% efficacy assumption.

It is important to discuss both hypotheses and to examine how they hold. As explained earlier, the constant potency ratio assumes that when both drugs are acting separately, the ratio between the dosages of marijuana and morphine are identical for any chosen effect level. Moreover, we recall that this hypothesis guarantees the linear shape of the isobole. To verify it, we introduce the following plot.

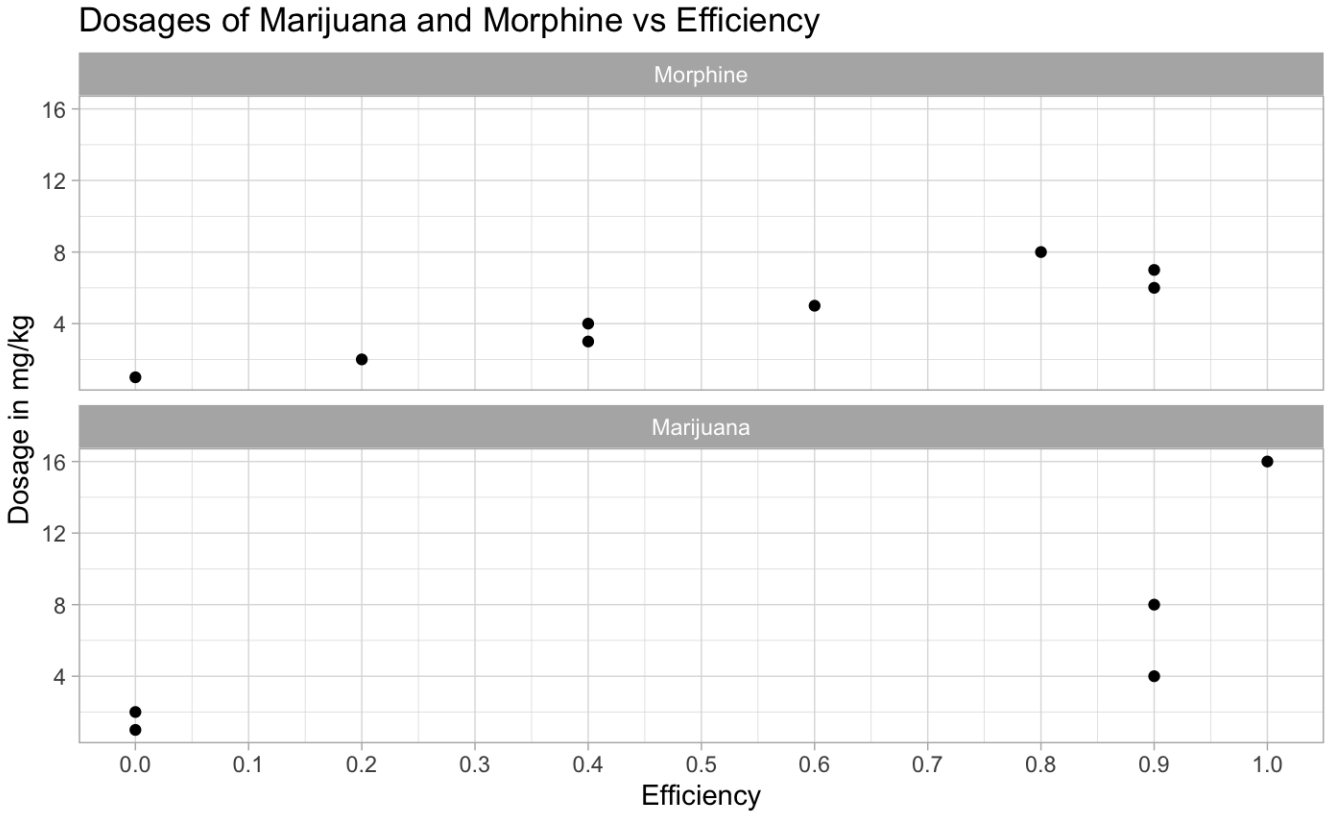


***Figure 4****:* Dosages of Marijuana and Morphine with respect to effect level on mice.

Upon observation of the marijuana plot, it becomes evident that there is a prominent gap in dosage and effect. Consequently, this gap makes it impossible to divide the dosage values of morphine and marijuana, which should be vertically aligned for verification of the assumption. Morphine and marijuana dosages only meet at effect levels of 0.0 and 0.9, which is insufficient to establish if the ratio of dosages remains globally constant or not.

Diché Consulting has decided to continue the use of a linear isobole despite the abovementioned issue. An overview of literature highlights numerous studies done on rodents to test the interactions between painkiller drugs, such as morphine, tramadol, other opioids, or cocaine. These experiments have employed similar methodology to the Company wherein they also utilize the linear isobole method. Thus, we believe that if the experiment were to be completed as detailed in our recommendations, the constant potency ratio could be verified.

The second assumption is the theoretical 50% effect level dosages for morphine and marijuana. These values have been extrapolated from the dose-response curves. For morphine, the experimental dosages tested allow researchers to record a detailed progression of the effect level. This allows to situate the 50% effect level inside a 1 mg/kg wide interval, between 4 mg/kg and 5 mg/kg. However, this is not the case for marijuana. Between doses of 2 and 4 mg/kg of marijuana, the effect level goes from 0.0 to 0.9. This represents an interval of 2 mg/kg, which is twice as wide as for morphine and the change in values of the effect level is much more drastic. Without more detailed data for marijuana dosages between 2 and 4 mg/kg, Diché Consulting assumed that the 50% effect level

should be around 3.0 mg/kg. However, this assumption is tenuous given the limited data for intermediate effect levels, as we do not know how the function grows from a null effect to a nearly total effect.

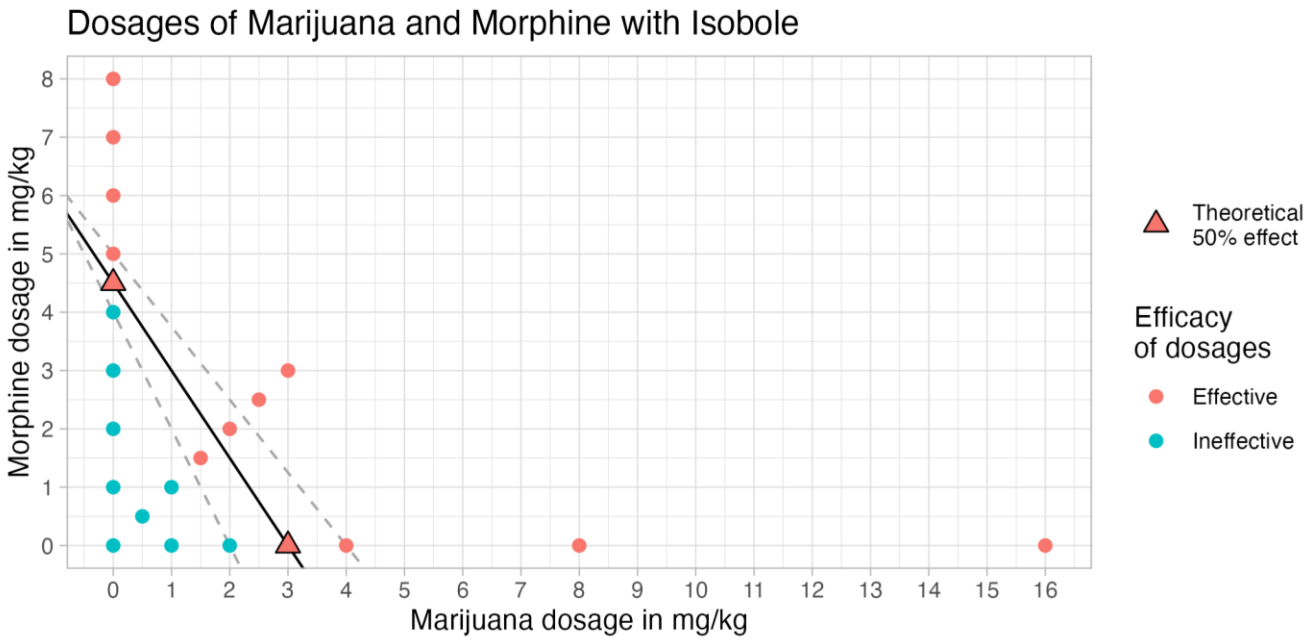


***Figure 5****:* Isobole with intervals of variation between which the theoretical 50% efficacy dosages could be placed.

Moving the dosages represented by the triangles in Figure 5 could drastically change the isobole's position, which would then determine if 1.5 mg/kg of marijuana and morphine are synergistic. Finding accurate 50% efficacy dosages is crucial to conclude the presence of synergy. To address these problems, Diché Consulting has prepared recommendations for completing and refining the experiment.

## Recommendations

The statistical weaknesses of this experiment principally stem from one flaw: the inadequate dosage choices for experiments with marijuana only. We display the effect levels for each drug below.

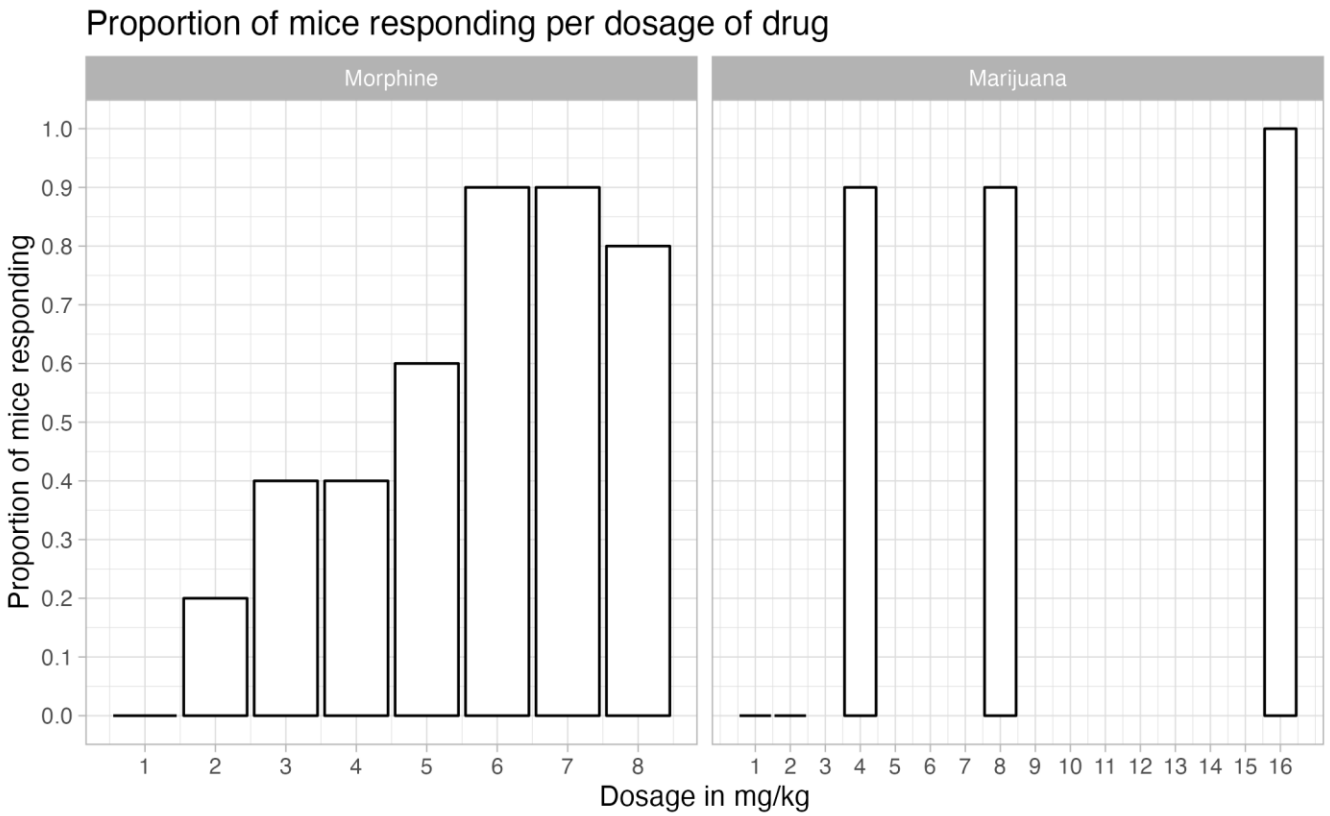


***Figure 6****:* Bar charts of effect level per dosage of morphine and marijuana.

To accurately determine the 50% effect level, it is necessary to obtain a bar chart which grows smoothly without too many abrupt gaps, especially around middle values. Unfortunately, this is not the case with marijuana, which lacks a bar chart that resembles that of morphine. To address this, it is crucial to test values of marijuana which are between 2 and 4 mg/kg to obtain a more total and cultivated result. **Diché Consulting's first and main recommendation is to supplement the experiment with at least four trials for values between 2 and 4 mg/kg of marijuana alone.** This would verify the constant potency ratio assumption and determine a more accurate 50% effect level for marijuana. Furthermore, this would also insinuate that a smaller minimal effective dosage for marijuana under 4 mg/kg would be discovered. This represents potential cost savings for the Company. Diché Consulting emphasizes that the experiment's worth is minimal without this additional step, as we regard it as **essential**.

A second recommendation would be to attempt determining a more precise 50% effective dosage for morphine too, even though the interval in which it is situated is smaller than for marijuana and the behavior of the dose-response curve of morphine is better known, as effect values increase more smoothly. In addition, Diché recommends exploring the possibility of identifying other synergistic dosages beyond those tested, which were limited to identical amounts of morphine and marijuana. This could involve testing different combinations of dosages, such as 1 mg/kg of one drug and 2 mg/kg of the other. Such an investigation would be particularly valuable if one drug is cheaper than the other.

## Conclusion

The first objective pertained to determining the minimal effective dosages for marijuana and morphine. Throughout analysis, Diché Consulting has concluded that these dosages would be formulated as:

- 4.0 mg/kg for marijuana alone;

- 5.0 mg/kg for morphine alone;
- 1.5 mg/kg of marijuana and 1.5 mg/kg of morphine for both drugs.

If the Company were to implement the recommendation about marijuana dosages, the first amount listed of 4 mg/kg could be reduced.

The second objective was to establish if there is synergy between marijuana and morphine. The dosage of 1.5 mg/kg of each drug has a potential to be synergistic. Unfortunately, the current state of the experiment does not allow us to conclude that there is synergy. Diché Consulting has detailed how to complete the experiment with marijuana dosages so that synergy can effectively be determined. We strongly advise the Company to act, as these few steps would give the experiment a sturdy foundation while uncovering a new advantageous minimal dosage. From our analysis, the Company has already implemented a correct methodology and does not need to change its experimental process. By adding a few more wisely chosen trials, this could greatly benefit the experiment, boosting accuracy and improving general comprehension.

## Appendix

This appendix details how the linear isobole is directly derived from the constant potency ratio assumption. Let *A* and *B* be the 50% effective dosages for two drugs $D_A$ and $D_B$. We suppose that the potency ratio between the two drugs is constant. Thus, we can define the ratio to be

$$R = \frac{A}{B}$$

If the effect level varies, *R* will remain unchanged as it is a constant. This constant ratio allows us to convert dosages from one drug to the other. This is useful because as it stands, summing a dosage *a* of drug $D_A$ with a dosage *b* of drug $D_B$ is incorrect, unless we convert either one to a dosage for the other drug. The following conversions can be done:

$$a \quad \leftrightarrow \quad \frac{B}{A}a = R^{-1}a$$
$$b \quad \leftrightarrow \quad \frac{A}{B}b = \quad Rb$$

The first conversion allows to transform a dosage $D_A$ into its equivalent dosage of $D_B$, while the second conversion does the exact opposite. We will use the second conversion to transform *b* in its equivalent dosage of drug $D_A$. It is important to discuss the following: the isobole is exactly the set of dosage pairs *(a, b)* which achieve a 50% efficacy level together. Moreover, under the assumption that both drugs act independently, with no interaction, the 50% efficacy level is exactly the sum of the efficacy levels of given by dosages *a* and *b*. As we have discussed, for the sum to be correct, the appropriate conversion must be made. Finally, for *(a, b)* to belong to the isobole, the sum of their dosages must be equal to dosage *A* (if we convert the two dosages to dosages for drug $D_A$, otherwise it will be *B* if we choose drug $D_B$). What we have explained translates into the following equation:

$$a + [b]_{D_B} = a + \frac{A}{B}b = A$$
$$\Leftrightarrow \frac{a}{A} + \frac{b}{B} = 1$$

This is exactly the linear isobole equation Diché has used. Dosages *a* and *b* represent coordinates *x* and *y* on a graph, and the above equation traces a line between points *(A, 0)* and *(0, B)* as we have done.

## Bibliography

Tallarida, R. J. (2011). Quantitative methods for assessing drug synergism. *Genes & cancer, 2(11)*, 1003-1008.