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TOXIC MIXTURES A PROBLEMATIC CASE OF TOXICITY QUALITY INDEXES

Abstract: *The CLP and REACh regulations recommend quality toxicological indexes for the chemical mixtures that must be introduced to the market. Until few years ago the experimental toxicity was based on testing only one substance at a time. However, in our daily life we use various chemical substances and their combination can increase or decrease the final toxic response. Mathematic models that forecast the combined toxicity have been developed already by the late 1800's nevertheless few studies have investigated in this sector. The present study collects the most interesting experiments of combined toxicity and tries to distinguish among the different types of toxic mixtures, for this reason the main mathematical models of this sector are examined and analysed.*
Keywords: *Combined toxicity, mathematical models*

1. INTRODUCTION

Toxicity and toxic substances are widely used terms; however, the distinction among a toxic and not toxic substance is not so easy. Paracelsus is considered the father of toxicology and he had said that all substances can be toxic, "*the dose makes the poison*". According to this aphorism the toxicity tests till some years ago were focused to measure the toxic dose of one substance at a time. Nevertheless it is almost impossible to find one substance alone even in simple samples. The situation is becoming more complicated if we consider the definition of a chemical mixture given by the US-EPA "*mixtures will be defined as any combination of two or more chemical substances regardless of source or of spatial or temporal proximity*" (EPA, 1986).
The presence of two or more substances can increase or decrease the toxic response according to their type of interaction [1]. The final toxicity can be enhanced when we have synergistic action, decrease when we have antagonism (the case of antidotes), be the same as the addition of the two toxic responses or be potentially enhanced when one substance by itself is not toxic but becomes toxic with the presence of another.
For avoiding expensive toxicity and repetitive tests scientists use mathematical methods in order to understand the type of actions with the simple dose-response relation [2].

2. INDEPENDENT ACTION OR CONCENTRATION ADDITION?

2.1 Basic assumptions

The independent action it is also called zero interaction or response addition because the final toxic result is the same as if the two substances were acting at the

same organism but at different time. Two agents that have no common intermediate lesion in their action will be non-interactive [3]. The independent action of two or more substances probably is caused by their different chemical pathways in the organism [4]. Concentration addition on the other hand is when the substances act in a similar way and their toxic results can be summed [5]. Hermens believes that when two substances act independently may have minor toxic result than in the case of the concentration addition [6]. There are many mathematical models for predicting the type of the toxic mixture but most of them are based on the concepts of concentration addition and response addition. The models of concentration addition are used mainly for the substances that they have common type of action in the organism and the models of response addition for those that act independently [7]. Generally the mathematical model is based on the toxicity of each substance of the mixture, taking into account their percentage [8] and describes the action without any theoretical interaction.

Once we can decide on which type of models, concentration or response addition we can be based on, we proceed by choosing the particular mathematical rule/model. And we compare the results of the model with the results of the toxicity tests. If the model gives the same toxic result mathematically as the toxicity test then we have simple addition (concentration or response addition). If the model gives more toxic result than the real toxicity test, we have antagonism. Synergy is the case when our mathematical model result is less toxic than the real mixture toxicity.

3. TYPES OF TOXIC ACTION

3.1 Synergy

The combination of two or more substances may be synergistic if the result of the combination is more toxic compared with the result that we would have if we would experiment the toxicants separately and one at a time. The synergistic action increments the final toxicity. Studies in phenol, pent phenol and dinitrophenol indicate that their combination becomes more toxic than expected [9]. An other example of this type of action is among vinolelbin and γ-linoleic acid [10].

3.2 Antagonism

Antagonism is the general case of the antidotes, when one substance opposes to the action of the other. This probably happens when the substances inside the organism give as a product a non toxic substance, or their actions may be opposed i.e. one increases the blood pressure and the other decreases it. An other type of antagonism is when the presence of one substance decreases the time of the permanence of the other inside the organism.

An illustrate example is when an organism is poisoned with Arsenic and Mercury, their ions can create chelates with 2,3-dimercapto-1-propanol and in this way to cage the toxic action of the metal.

33. Potential enhance

When a substance is not toxic itself but with the presence of an other it becomes toxic i.e. 2-propanol is not toxic at liver but can enhance the toxicity of carbon tetrachloride.

4. MODELS BASED ON RESPONDE ADDITION

4.1 Probit Analysis (1934)

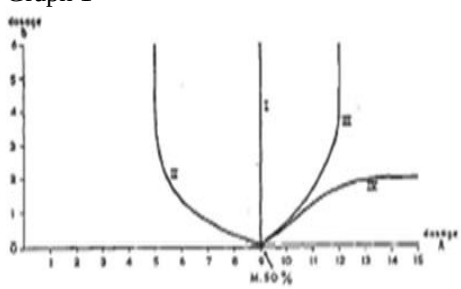
Probit analysis as term is initially used by Bliss (1934) for converting the mortality into probability unit, *Probit*. Nowadays it is also used in other scientific fields as in mathematics and statistics. Bliss distinguishes among independent action and concentration addition, he is referring to the first type of action as two substances A and B act in the same organism with

different chemical pathways.
The mathematical rule of independent action is the following equation 1:
Eq. (1) pc=pa+pb-papb
Where the pc is the combined toxicity
pa is the probit of the toxic response given by the a substance and pb the probit of the toxic response by the b substance.
The method is based on the fact that the curve dose-mortality is an asymmetric clockwise curve slightly bigger at its right quarter. We transform this curve into sigmoid and we convert the toxicity doses into logarithmic values and then into probits. In this way we obtain one line dose/mortality but we may loose important information after all these transformations.

4.2. Isobole

The term isobole has been used initially by Loewe και Muischnek , 1926 but on 1964 Tammes has borrowed this term for developing his predictive model of toxicity of mixtures [11]. The method is based on a graphic representation. Tammes distinguishes among toxic substances that act simultaneously and substances that act consequentially, respectively shown on graph1 and graph 2.

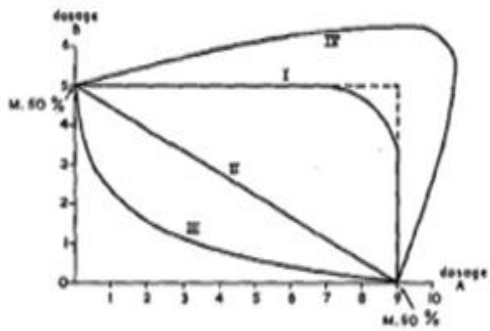
Graph 1



The horizontal ax shows the dosage of substance A and the EC50 of this substance, on vertical axes the substance B. The I line shows the result of both substances and it is a clear independent action, if there is not any interaction among the two substances then the EC50 will not change even if we add the substance B. In case of synergy the effect dose will be the same but with less substances either B and A, it is the curve II. The curve III shows the case of antagonism because with both the substances we obtain the same toxic result, and the curve IV shows the total blockage of the toxic action of substance A by the B.

Tammes has invented another diagram (graph 2) when two substances act simultaneously.

Graph 2



The line I is the case of independent action, we obtain two lines each one for the EC_{50} point of the substance A and B, the point of their intersection is where both A and B will kill independently the half of the population. The final mortality will be 75% according the sum of the mortalities, if the first substance kills the 50% of the population then the other kills the 50% of the population left. Tammes through this diagram distinguishes the independent action with the concentration addition, the line II is the case of the concentration addition and shows clearly that we can add the two substances in different analogies and we obtain the same result, as one substance can substitute the other. The III line is the case of synergy because fewer amounts of the substances give the same toxicological endpoint and the curve IV is the case of antagonism.

Tammes for his experimental models has used mixtures of parathion and Malathion, concentration addition case. For the synergy results he used amitrole

and atrazine.

The biggest problem for using this method is that it needs too many data [12] [13]. This is one of the reasons that this model has been used widely for investigating the toxicity of insecticides since there are insects in abundance. The method is easily interpretable thanks to the graphic representation but on the other hand it is not so easy estimable optically in which curve our data belong to, i.e. if our toxic points result in the middle among the I and II curve, then we don't know if our case is the synergistic or additive one, for this reason the method is only qualitative and can't be used for obtaining quantified results.

4.3. Probability

The method based on probabilities is developed by Koungolos in 1992. It is based on independent action and to the rule of probability of independent events. We transform the toxic concentrations in probabilities and then we apply the sum of the events as shown on equation 2.

Eq. (2)

$$p(C1+C2)=p(C1)+p(C2)-p(C1)p(C2)$$

where p(C1+C2) is the contemporaneous exposition to two substances, p(C1) and (C2) are the toxic endpoints of the two substances separately and the p(C1)p(C2) is the probability of the survivor after the exposition.

It is very flexible, without difficult calculations. It's well applicable in cases of numerous samples, as microtox tests, where the organisms are millions and the theory of probabilities can be easily applied. It has been applied for the investigation of the toxic action among heavy metals, (Cr, Cd, Ni) to *sacharomyciae* and agrochemicals (Methylparathion, Atrazine, Lindane, to daphnids (*Daphnia magna*), algae (*Selenastrum capricornutum*) and to bacterial (*Vibrio fisheri*), [14].

5. MODELS BASED ON CONCENTRATION ADDITION

5.1 QSAR Quantitative Structure-Activity Relationships

The purpose of the study QSAR in toxicology is for estimating (predict) the toxicity based on the structure of the molecules. We choose substances that all act at the same way. The first generation of QSARs were developed for predicting the toxicity of chemicals when there were not so many data and additionally most of them were not experimental [15].

For a QSAR study we must first divide our chemical substances onto classes, this step is not so easy due to the fact that there are many factors that make similar or dissimilar some molecules, i.e. phenyl alcohol can be classified either as alcohol or as aromatic substance. The second and most crucial step of this method is to select the descriptors. Latest years of research have provided thousands of descriptors and there are many software or data basis available for this purpose, i.e. DRAGON software with more than 4.500 descriptors. It is important to select the appropriate ones because the regression among the toxicity should have logic correlation with the structure, QSAR researchers should know very well the data they treat. Because it is difficult to select among thousands of descriptors there are mathematical methods, *chemometrics* that may facilitate this step but we cannot be based only to the science of mathematics. The personal evaluation of the descriptors and the opinion of the expert is necessary.

The first who used QSARs for mixtures was Konneman on 1980 on his study of "*structure-activity relationships and additivity in fish toxicities of environmental pollutants*" investigated 50 industrial pollutants [16]. According to the

results most of the predicitive toxic results were comparable to the real toxic data.

6. CONCLUSIONS

There are many models in the field of combined toxicity and every model has its own approach, this means that still we cannot give a final solution, appropriate for all substances and all toxic experiments. Generally the models are divided into two big categories: the concentration addition and the response addition but not all mixtures do act clearly with the one or the other mechanism.

There are also particular cases where these models cannot be applicable. All the models based on concentration addition cannot be used when the sum of the two toxic responses is bigger than the 100% (Folt et al, 1999). The model of probability cannot be used in the case of hormesis, and the isobole diagram can't treat more than two substances. The model given by Bliss calculates the action of the mixture taking into account that the two substances are given sequentially to the sample and cannot be used for testing contemporaneously. This case is particularly problematic in toxicology because if we consider the substances in sequence then our second toxicant act to the organisms that are left alive by the first. The QSAR method has the advance to be able to treat substances without experiments and it is proposed by the REACh regulation, but it needs expertise in the field of chemometrics and toxicology. The problem becomes crucial nowdays because toxicological indexes are demanded by the regulations even for mixtures, as important index of quality for the final product.

The truth is that few mixtures act completely independently and few mixtures act in a similar way, probably the main problem of mixture toxicity is based on the toxicity as science itself, because we are based on measuring only the concentration of the substance and the mortality. *The mistake is that we look at the test organism as a black box* [17].

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