User-defined transforms – useful feature of SigmaPlot

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April 2009

SigmaPlot´s unique feature of using custom-made transforms not only allows various convenient calculations with data in a worksheet but also enables the construction of theoretical curves, for example. The latter was an argument for using this program in dose-response curve studies in the field of pharmacology [1, 2] first and in toxicology [3], more recently. To illustrate how useful working with user-defined transforms is, we want to give a few examples here.

The Worksheet functions we use most are “col” and “cell” to read data from the worksheet and to put the transform result into a specified column or cell. The latter can be done in two ways as exemplified below.

“Absolute” and relative effects

In dose-response curve (DRC) studies we sometimes observe that the minimum of a curve starts at higher values than zero and/or the maximum is below 100 %. For certain operations and better visual comparison of curves it is an advantage to transform absolute into relative effect values between 0 and 100 %.

The transform below*, shown for substance A, copies original y-values into the next column, first. Relative effects of A (relA) are calculated from the maximum (max) and minimum (min) of a Standard 4-parameter logistic curve, run previously, and the original effect values. Relative effects of A (relA) then are placed into column 2 by the “put into” function.

```
;Relative effects Std-Logistic

col(3)=col(2)

maxA=cell(21,2)
minA=cell(21,1)
deltaA=maxA-minA
delta3=col(3)-minA
relA=(delta3/deltaA)*100
put relA into col(2)
```

* Please note: the syntax for the transforms below are for standard UK & US settings; standard continental users would want to substitute comma decimals and semicolon list separators

Absolute and relative effects are illustrated by DRCs of ethyl bromoacetate (EBAC) at 15, 30, and 45 min of incubation in Fig. 1 and Fig. 2. It is evident that the EC50 is reduced at longer exposure durations from 1.03, 0.49, and 0.33 mg/L respectively, regardless of the type of presentation. Fig. 1 shows an increase in the bottom of the DRC from 3.8 to 16.6, and 27.4 % as the time of testing increased.

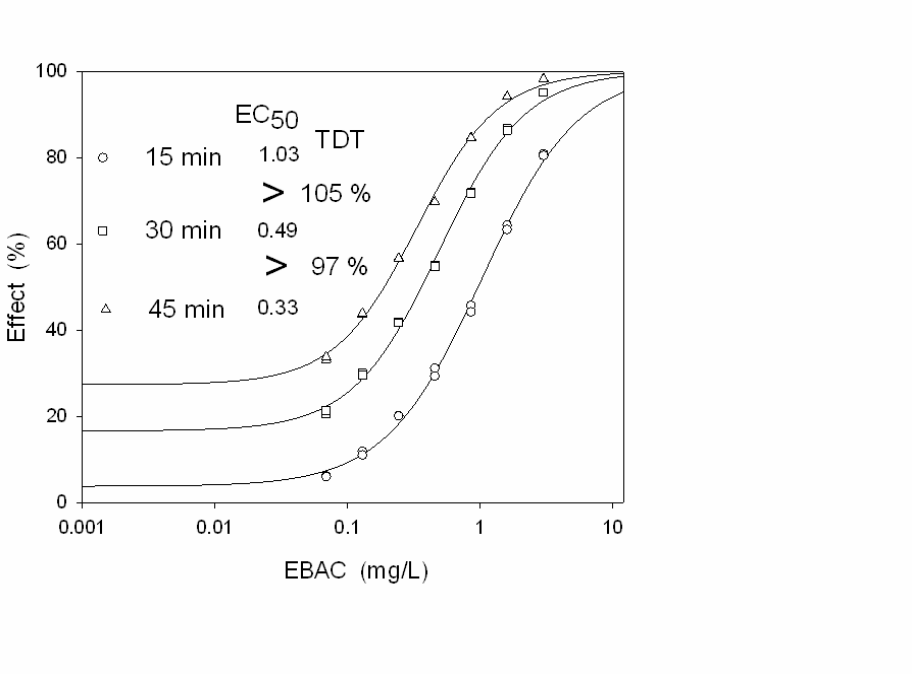

Fig. 1 DRCs of EBAC at 15, 30, and 45 min incubation, EC50s and time-dependent toxicity (TDT). "Absolute" effect values

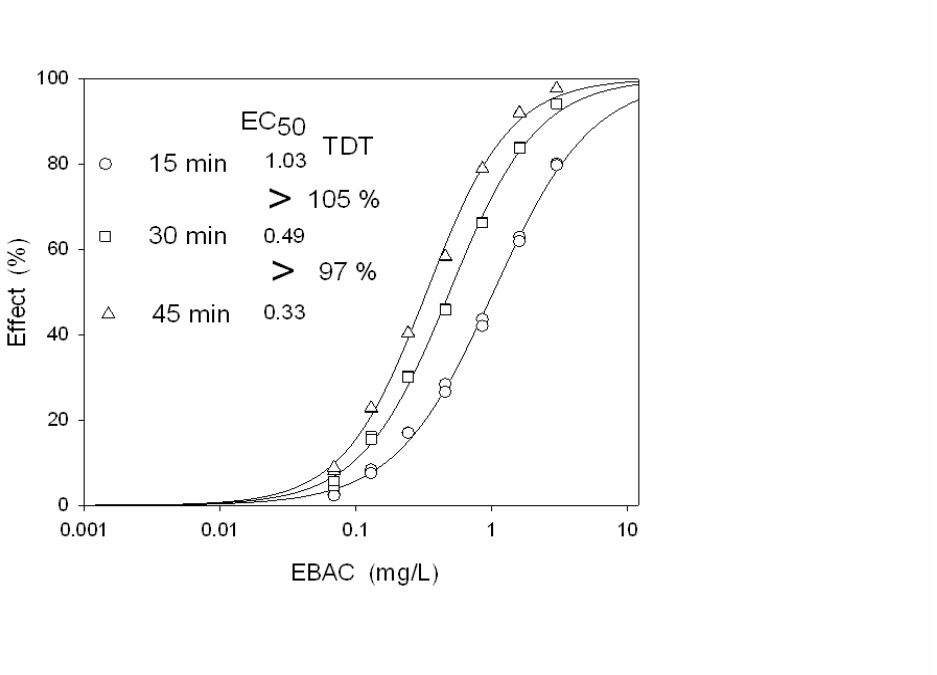

Fig. 2 analogous to Fig. 1 but with relative effect values

This example was chosen because it also illustrates time-dependent toxicity, described below.

Time-dependent toxicity

Irreversible action of toxic agents increases with time of exposure.
Calculation of time-dependent toxicity (TDT) [4,5] is based on the respective equation
E = c * t
where E is the effect, c the concentration, and t the time of exposure. From this relationship it follows, for instance, that doubling the time of exposure leads to the same effect when the concentration c is reduced to 1/2. Time of incubation in our experiments was 15, 30, and 45 min, respectively. Concentration c is the EC50 determined by the 4-parameter logistic function and listed in cells (21,3 – 23,3) before running this transform. TDT is expressed as percentage of maximum calculated by the equation referred to above.

```
'Time Dependence col 21-23

c15=cell(21,3)
c30=cell(22,3)
c45=cell(23,3)

TDT30=100*((c15-c30)/(c15*1/2))
put TDT30 into cell(22,6)

TDT45=100*((c15-c45)/(c15*2/3))
put TDT45 into cell(23,6)

TDT45x=100*((c30-c45)/(c30*1/3))
put TDT45x into cell(23,7)
```

Fig. 1 and 2, respectively, show the calculated TDT values at 30 min vs 15 min (105 %) and at 45 min vs 30 min (97 %), demonstrating about 100 % time-dependent toxicity [6]. The three DRCs of EBAC exhibit a slight increase in slope over the time of incubation from 1.20 to 1.41, visually evident in Fig. 2.

Effects in combination

Fixed-dose experiments

We are often interested whether the effects of an agent A are affected by a certain, (fixed) dose of an agent B, with respect to synergism (or potentiation) or antagonism. When B exerts an effect on its own similar to that of A we can compare, for instance, the experimental dose-response curve (DRC) of A alone and in the presence of B with theoretical curves of two models of combinations, dose-additivity and independence.

Dose-additive combinations

are characterized by an effect in combination as a result of a certain dose of A in the presence of B. This effect is expressed as caused by an additional dose of A which is equieffective with a given dose of B. This type of combination considers B to be acting like A, for example by binding to the same receptor. Hence, as a general rule, agents that show a common site of action will give a combined effect not different from dose-additive.

We can apply the following transform. There, xA and yA represent x/y-values of a curve A, and xB1eq and xB2eq are two fixed doses of B which are equieffective with a specified dose of A.

```
; A+Bfix Dose-additive curves

xA=col(30)
yA=col(31)

xB1eq=cell(29,1)
xB2eq=cell(29,2)

xADD1=xA-xB1eq
put xADD1 into col(35)
xADD2=xA-xB2eq
put xADD2 into col(36)
```

The additive curves are then defined by
xADD1 and yA and
xADD2 and yA, respectively.

Interesting examples are combinations of two agonists binding to one and the same receptor [2]. Even more interesting is the effect of a full agonist in the presence of a partial agonist with DRCs shifted to the right parallel to the additive curve [7] which can conveniently be constructed by a user-defined transform.

Dose-additivity is exemplified here by ß-aminopropionitrile (ßAPN) alone and in the presence of a fixed concentration of ßAPN, i.e., a “sham combination” in fact (Fig. 3). Whereas the experimental DRC of ßAPN in combination with itself is close to the dotted line of dose-additivity [8], this phenomenon is not observed in Fig. 4 with ßAPN in the presence of a differently acting agent penicillamine (PNC) under otherwise identical experimental conditions [9]. Here, the experimental curve for the combination lies about in the middle between the dose-additive curve and the theoretical curve for independence, described below.

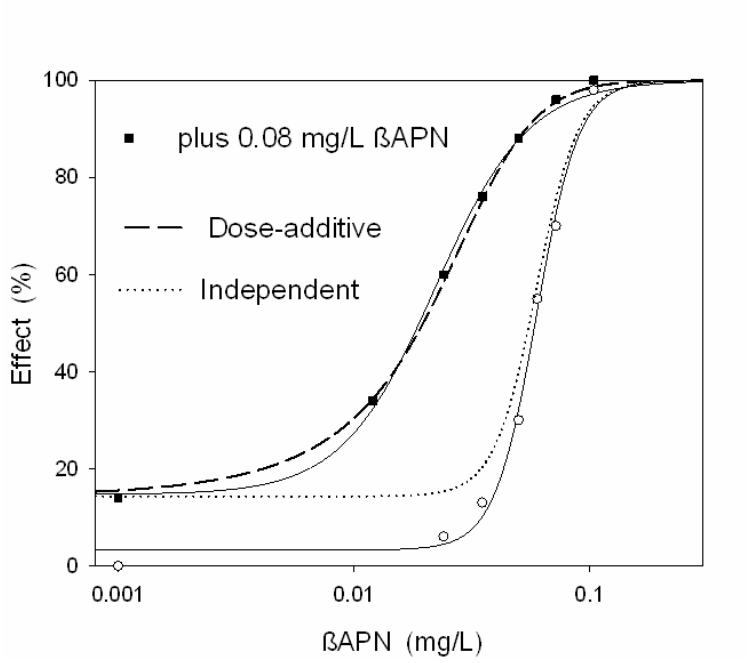

Fig. 3 DRCs of ßAPN alone and in the presence of a second sample of ßAPN. Example of a dose-additive combination

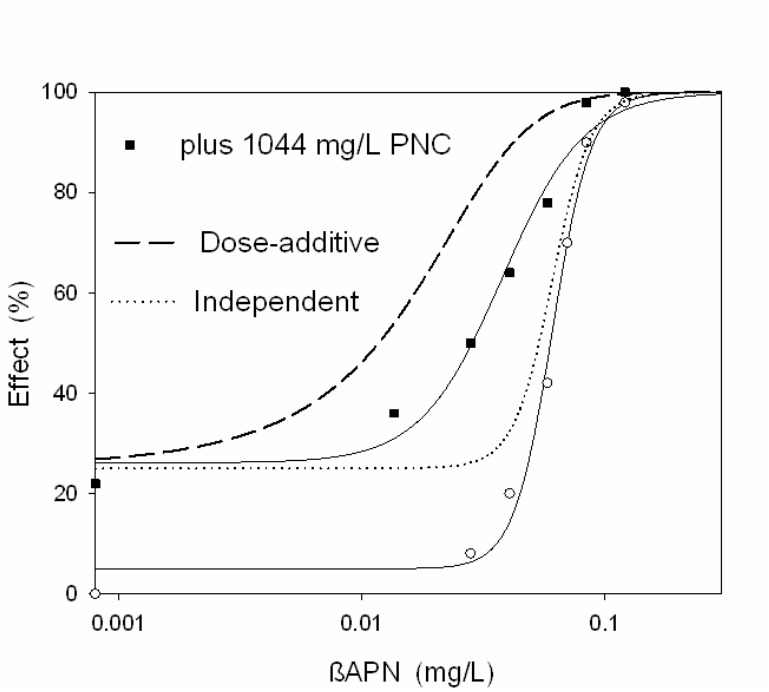

Fig. 4 DRCs of ßAPN alone and in the presence of a fixed concentration of PNC. Example of a non-additive combination

Independence

This type of combination assumes that the effects of A and B are independent in action. Again, a user-defined transform can nicely calculate curves of independence.

When we analyze DRCs of A alone and in the presence of a fixed dose of independently acting B, the ED50 of A as well as the curve slope remains unchanged, hence, the relative effects remain unaffected. This combination can also be seen as relative effect-addition.

Independent effects expressed as percent of maximum of agents A and B can be calculated by

A+B = A + (B (100-A) / 100)

Given that percentage effects are y-values of curves in specified columns, for example:
xA = col(30), yA = col(31),
yB1 = cell(32,1), yB2 = cell(33,1),
a rather simple transform will give us the x/y-values of independent curves of

A+B (in columns 37 and 38). Note that the transform below calculates two curves which are corrected for eventual background of A = cell(21,1).

```
;A+Bfix Independent curves

yA=col(31)
yB1=cell(32,1)-cell(21,1)
yB2=cell(33,1)-cell(21,1)

ind1=yA+(yB1*(100-yA)/100)
put ind1 into col(37)

ind2=yA+(yB2*(100-yA)/100)
put ind2 into col(38)
```

Independent effects in combination can be calculated for effects expressed as a fraction of maximum by the equation

$$A+B = A + B -(A * B)$$

Note that curves of independent effects are characterized by unchanged EC50s and slope values of A.

Summary and Conclusions

Among other things, described above, user-defined transforms, especially in DRC-studies, can give valuable hints regarding the site of action of agents tested.

Common or different site of action?

From biochemistry and physiology to pharmacology and toxicology DRCs are a valuable tool in research, especially for, but not limited to, discerning between common or different action of the agents [10] under study. The use of theoretical DRCs in fixed-dose and in mixture experiments has given us insight into mechanisms of drugs and toxic agents. Another user report deals with mixtures.

A common mechanism among agents requires that their effect in combination is dose-additive or comes close to it if additional mechanisms come into play. Independent effects can occur with differently (and reversibly) acting agents, e.g., with neuroprotective agents [11] or with certain drugs in combination [12].

Effects greater than predicted by additivity and independence

User-defined transforms such as those described above are also advantageous for describing and interpreting effects greater than dose-additive or independent. It is generally agreed upon that greater effects than expected by the theoretical model of dose-additivity represent synergism. DRC-studies also allow a uniform characterization of potentiation [13, 14], reflected by an increase in potency of an agent A caused by B; characterized by a shift of the DRC´s ED50 to lower concentrations, as illustrated by Figures 5 and 6, characterized by the dose ratio (DR) = 13 (Fig. 5) and 59 (Fig. 6).

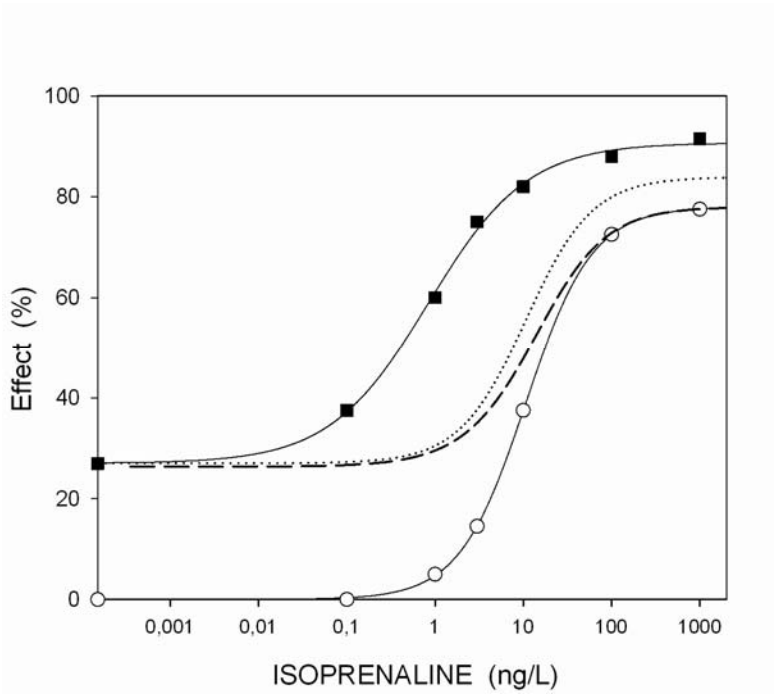

Fig. 5 DRCs of Isoprenaline alone and in the presence of a fixed concentration of theophylline [2]. Example of a potentiated combination.

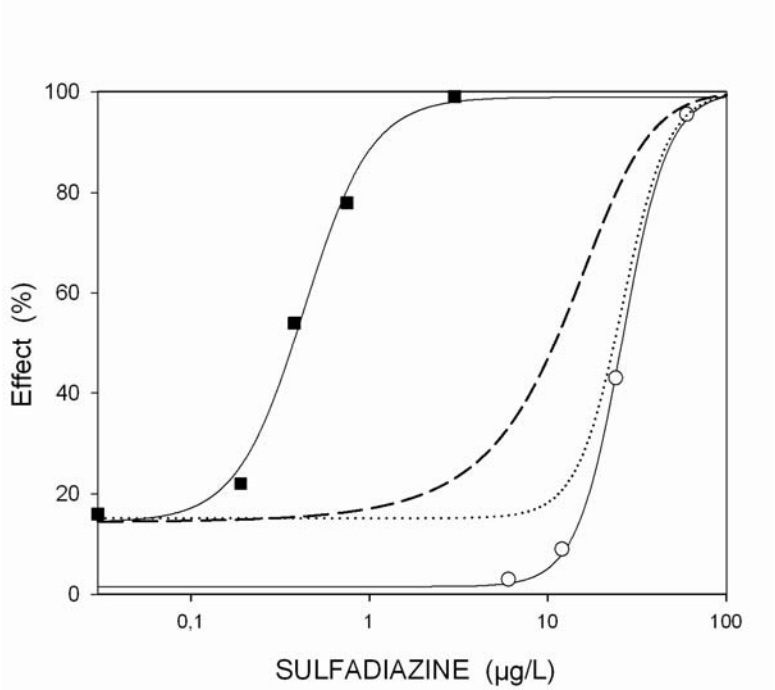

Fig. 6 DRCs of Sulfadiazine alone and in the presence of a fixed concentration of Pyrimethamine [15]. "Classical" example of a potentiated combination.

Potentiation in these two examples can be explained by different actions in sequence: Adenylyl cyclase activator plus a phosphodiesterase inhibitor (PDE-inhibitor) in Fig. 5, and two agents blocking the synthesis of tetrahydrofolate (THF) sequentially in Fig. 6.

Different experiments with numerous agents, drugs and different toxic agents revealed that the relationship between additivity and independence differs. In experiments with agents showing rather shallow DRCs (mainly in physiologiy and pharmacology) independent effects are greater than additive, for which Fig. 5 is a good example. Isoprenaline shows a curve slope = 1.15. With other agents (mainly in toxicology) additive effects are greater than or about equal with independence, depending on the steepness in slope of DRCs. This fact is exemplified in Fig. 6 with Sulfadiazine exhibiting a slope = 3.4.

Literature

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