A NEW APPROACH TO DIFFERENTIAL TOXICITY

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INTRODUCTION

The agents currently in clinical use for cancer chemotherapy produce toxic effects in the host which are often the limiting factor in the use of the agent. In preclinical screening for new agents the present emphasis is on indices, such as percentage inhibition, which are measures of efficacy against the tumor (e.g. "tumor toxicity") in most cases. While this emphasis is appropriate in primary screening, it is less appropriate in secondary testing, where the task is to intercompare different agents. It has long been recognized that tumor toxicity relative to host toxicity would provide a more plausible basis for intercomparison of agents than the tumor toxicity alone. While indices of relative toxicity date back to the turn of the century (and the work of Dr. Paul Ehrlich) there are numerous conceptual, technical, and practical difficulties with the various indices that have been developed. The purpose of this paper is to suggest an index of toxicity differential which avoids some of the difficulties encountered with previous indices.

This new index will be considered in the context of a particular experimental situation--laboratory animal screens are described more fully in another paper (3). The main features of this experimental situation were: 1) for each animal there was a separate index of tumor toxicity (tumor diameter) and of host toxicity (animal weight change*); 2) the experimental unit was a set of five animals. The average tumor diameter and the average weight change for the set constitute the basic data here; 3) each trial of an agent consisted of separate animals at 4-8 dosage levels (including a control or zero dosage level); 4) for each agent there were three such trials and usually the three trials were started within a period of one month; 5) twenty agents were tested over a twelve month period (one of the "agents" was food restriction); 6) the average tumor diameters were measured at two weeks on tumors produced by intramuscular inoculations of $1 \times 10^7$ Nelson ascites tumor cells into the right thigh of Swiss albino mice; diameters were measured with calipers in two perpendicular diameters; 7) the average weight changes were determined by weighing mice before the tumor inoculation and at one and two weeks; the one week data were used in the present calculations since in many cases the hosts regained, in the course of the second week, the weight loss caused by treatment. This was a secondary test so that nearly all of the agents had already passed through primary screens. Whether or not the new index would be of value in other experimental situations would have to be determined by empirical investigation but the potential area of application would include both laboratory and clinical testing.

CONCEPTUAL BASIS

The conceptual basis of the Toxicity Differential Index (henceforth abbreviated as TDI) can best be explained by considering what happens when a plot is made of tumor toxicity against host toxicity for several agents. Such a plot is shown in Chart 1 for three agents: food restriction, 6-mercaptopurine, and cytoxan. Although the points in Chart 1 represent actual data, some of the experimental curves are considerably more erratic than the ones shown here.

In interpreting Chart 1 we can start with the right-hand point on these curves, which represent the control values. The fact that the curves do not all start at the *This index of host toxicity was used instead of the mortality of the hosts since, at lower doses of the drugs, animals do not die but lose in body weight. At toxic dose levels there is a correlation between the two indices.
same point merely indicates that in this screen, as in any other, we must reckon with the inherent variability of the process.

Now let us focus on the food restriction curve (the flattest one). We observe that when the intake is reduced to 4 gm/day (the point next to the control value) the average tumor size is somewhat reduced and so is the average weight gain. As the intake is further restricted there is an incremental tumor toxicity corresponding to the incremental host toxicity. In most of these experiments the dosage range of the agent was extended to the point where the animals began to die in the first week.

The TDI has as its conceptual basis this notion of the increment in tumor toxicity for an increment in host toxicity. In other words it corresponds to the slope of the curve at a given point (which corresponds to a given dosage level). We would be looking for agents where this slope is large, where a small change in host toxicity would be accompanied by a large change in tumor toxicity. For an "ideal" agent we would have a nearly vertical slope. The tumor diameter would rapidly decrease while the weight change would be almost unaffected. For a poor agent the slope would be almost horizontal so that there would be increasing host toxicity with little effect on the tumor. Hence, the slope would be a simple and natural index of tumor toxicity relative to host toxicity.

The striking feature of Chart 1 is that the curves are very nearly straight lines. Similar plots have been used by previous authors, e.g., Pearson (1), and Sloboda (2) have noticed the linearity of obtained curves. When the curves are straight lines this means that the slope is constant. In other words, the slope does not depend on the dosage levels that are used to obtain the dosage response curves. If the curves are not linear, we would face the problem of choosing some particular level of tumor toxicity (or the corresponding dosage level of agent) and some particular level of host toxicity (or corresponding dosage level) and any comparisons between agents would then depend on these somewhat arbitrary decisions. As we shall see later there is a great variety in the shapes of the tumor toxicity and host toxicity dosage response curves. Consequently with one set of arbitrary decisions we could find Drug A superior to Drug B while another choice would lead to the conclusion that Drug B was superior to Drug A. This undesirable state of affairs is an inherent difficulty with the usual indices, including Ehrlich's chemotherapeutic index and Sloboda's modification. We do not get out of this difficulty merely by using the TDI. We only escape this difficulty if it is an empirical fact that the curves in Chart 1 are linear. The virtue of the TDI is that it enables us to exploit this favorable experimental situation.

When we plot the data as in Chart 1 and obtain reasonably straight lines the TDI is the slope of these lines. If the dosage levels cover the working range for the agents (extend, if necessary, to toxic levels), then the slope can be said to characterize the relative toxicity of the agent. In general, the greater the slope, the better the agent. Although the slope has a meaningful and useful interpretation in the sense that it measures the key property that the investigator is looking for in an agent, at this point it has two drawbacks. First, it is not dimensionless and therefore would depend on a unit of measurement used in the experiment. Second, the numerical value would be more meaningful if it were relative to some natural baseline. Both of these objections can easily be met by using the ratio of the slope for an agent to the slope for food restriction.

With food restriction we do not have host toxicity in the usual sense (e.g., as the action of a poison). A type of differential toxicity may be operating in that starvation may affect the tumor more than the host or vice-versa. Ordinarily, however, we would be looking for toxicity differentials over and above such starvation effects. In other words, if the agent were an antimetabolite which produced the same effect as starvation by interference with metabolic pathways, we would have limited interest in this type of differential toxicity. On the other hand if the antimetabolite showed the same incremental host toxicity as food restriction but a greater incremental tumor toxicity we would say that the agent showed differential toxicity. In the linear case, a slope ratio of unity would correspond to the first hypothetical antimetabolite whereas,
the second antimetabolite (the one which we would say showed differential toxicity) would have a slope ratio greater than one. Since the TDI actually used here will be the slope ratio, it does not seem semantically inappropriate to refer to those TDI's which are substantially greater than unity as indicative of a "Toxicity Differential".

TECHNICAL BASIS

In this section the TDI will be considered from a more technically oriented point of view. From the technical standpoint the problem is one of boiling down a fairly large number of observations into a single number capable of giving a useful characterization or description of the experimental situation. For each agent we have three trials with an average of about seven dosage levels per trial and two observations per dosage level or, a total of around forty observations. We would like to concentrate the information in these forty observations in a single observation (without excessive loss of information in the process). Moreover at the end we would like to be able to intercompare the indices for the different agents and to have available such statistical significance tests or estimates as would be needed.

In the path to this goal lie a number of road blocks -- difficulties that are inherent in the experimental situation of a secondary animal test. Some of these difficulties are illustrated in Chart 2. This chart shows some dosage response curves for tumor toxicity in two agents (food restriction and cytoxan) and two trials of each agent. If we wish to intercompare agents, then some of the difficulties indicated by Chart 2 are 1) variability in the starting or control observations, 2) some tendency for all the points to shift up or down from one experiment to another on the same agent, 3) irregularities in the course of the dosage response curves, differences in the shape of the dosage response curves. It will also be noted that the dosage response curves do not reduce to simple straight lines and hence the characterization of such curves would be quite complex. In the initial attempt at analyzing these data we used the tumor and host toxicity dosage response curves but the manifest difficulties soon forced us to abandon this line of attack. It might be noted that this approach leads directly to the conventional indices of relative toxicity and that we had originally intended to use a conventional index. In view of the troubles we encountered with the dosage response curves it came as quite a surprise when the method of Chart 1 yielded relatively simple curves. Chart 3 shows the corresponding host toxicity response curves. In this instance the host toxicity curves are fairly well behaved but for some other agents they were not.

Chart 4 shows a replot of the data in Charts 2 and 3. Chart 4 follows the same procedure used in Chart 1 except that instead of connecting the points the fitted regression lines are shown.

Since the word "regression" to many oncologists means a regression of treated tumors it should be clearly noted that in the following discussion the word "regression" has its technical statistical meaning. It refers to a specific method for calculating slope estimates. As can be seen from Chart 4 the slopes differ from one trial to the next and these differences are more striking than the irregularities within a trial. In other words the between trial variation tends to dominate the within trial variation and therefore we would prefer to base significance tests and confidence intervals on the between trial variance. Note that the shifts up or down that are so striking in Chart 2 -- and which are very troublesome if we try to use arbitrary tumor host toxicity levels -- are much less troublesome in Chart 4.

If the underlying curves of Chart 1 (or 4) are linear, then the slope can be regarded as completely characterizing drug performance so far as differential toxicity is concerned. In this event, the regression estimate of the slope extracts all of the relevant information in a trial and the average of the three slope estimates contains nearly all the relevant information in the three trials. Hence the forty or so original observations are boiled down to a single index without appreciable loss of information. If the underlying curves are non-linear or if the traditional indices are used, this efficient and succinct summarization cannot be achieved.
If plotted points in diagrams such as Chart 1 actually fall close to a straight line and if the corresponding line segment is reasonably long, then an eye-fitted straight line will not differ much from the regression-fitted line. Therefore, the bulk of the computational labor can be avoided by eye-fitting but little information will be lost. The same significance tests and confidence intervals will still be available with eye-fitting. If eye-fitting is difficult in a particular trial, it would be feasible to use regression only in the difficult cases. With this simplification, the computations for the TDI are easier than the corresponding calculations for the usual indices.

OPERATIONAL BASIS

The analytical procedure suggested in the preceding section will now be detailed:

1. For each trial of each agent a straight line was fitted by standard regression methods to the set of points obtained from the dosage response curves. As a check against numerical mistakes or unusual circumstances we would recommend that a plot of the data and fitted curves (as in Chart 4) be made.

2. The slope estimate (and the estimate of the within-trial variance of the slope estimate) was computed. If eye-fitting is used the slope of the fitted line would be estimated directly and there would be no within-trial variance estimate.

3. For each agent the average of the three slope estimates was calculated together with the direct estimate of the between trial variance. If pooling is appropriate (a matter which is discussed in Appendix I), the standard analysis of variance format for one-way classification is a computational shortcut. The same procedure would be used with either regression or eye-fitting.

4. The TDI, the ratio of the average slope for each agent to the average slope for food restriction was calculated. Corresponding confidence intervals were obtained by (a) estimating the error variance from the pooled estimates of between trial variation [e.g. the between mean square in the analysis of variance (ANOVA) table], (b) dividing by 3 to estimate the variance of the average slope, (c) taking this quantity as the estimate of $\sigma$ and putting $2\sigma$ confidence limits on the average slope (i.e. average $\pm 2\sigma$, average $\pm 2\sigma$), (d) dividing the limits by the average slope for food restriction.

This procedure led to the confidence interval graph for the agents that are shown in Chart 5. The agents have been arranged in order of increasing slope estimates and the chemical names are shown in Table I. Chart 5 answers most of the questions that we might want to ask about the relative toxicity differentials of the agents.

From the mean (central) values in Chart 5, we obtain a general picture of the toxicity differentials. We see, for example, that there is only a single agent whose TDI is less than unity (i.e. food restriction) although there are a couple of other agents whose TDI is close to unity. The largest TDI is 3.6 and there are five agents with TDI's of two or more. So we see that the performance of the agents under test covers a fairly wide range.

The next question is whether these differences in the TDI's mean anything or whether they merely reflect the large inherent variability in screening experiments of this type. To answer this question the confidence limits are useful. We note that for the top four agents there is no overlap of their confidence intervals with the interval for food restriction. This means that these TDI's are demonstrably different -- in the usual statistical sense -- from unity. There are three or four more agents where there is only a little overlap and these can be regarded as borderline cases. The top agent stands out from all of the other agents.

In interpreting confidence interval graphs of this type, a little caution is needed in stating probability levels. The nominal level here is 5 per cent but two
conflicting factors complicate the picture. For an individual comparison the no-
overlap rule is extremely conservative so that the actual level is closer to 1 per cent.
However, when we intercompare a number of agents and make a series of statements the
chance of making at least one false positive statement is substantially greater than
the nominal level. These two factors tend to cancel each other out if there are only
ten-twenty agents so that the actual probability level will be in the vicinity of 5
per cent (but not exactly 5 per cent).

There are other important qualifications on any statements. At best the state-
ment will hold for the tumor-host system of the screen and the results may or may not
carry over to other systems or to clinical applications.

Even for the same tumor-host system, protocol modifications may well change the
picture completely. For example, when the ascites form of the tumor was used in a paral-
lel experiment, the total packed cell volume against the weight change led to distinctly
non-linear curves. The analysis used here for tumor size could not be used for the
parallel data. For linear systems we might hope that the TDI would provide greater com-
parability of results in two different systems than could be attained with conventional
indices -- but an empirical study would be needed to see if this hope was realized.

DISCUSSION

Having considered the conceptual, technical, and operational basis of the TDI,
we will now discuss some of the advantages and limitations of the procedure. The
principal limitation of the methods used here is that they require a linear relation-
ship between the tumor and the host toxicity indices. The underlying concept of incre-
mental change in tumor toxicity relative to incremental change in host toxicity (a
differential in the mathematical sense of the word) applies also to non-linear situ-
ations but in such situations the technical simplicity is lost. Therefore in the
following discussion all of the statements require the qualification "in a linear
situation".

Chart 5 illustrates the advantages of the TDI for practical purposes. It shows
that the TDI enables us to discriminate rather well between the agents in the screen.
While the experiments that lead to Chart 5 require a fairly large number of animals they
are well within the range of practical screening. The numerical values of the TDI have
a simple and straightforward interpretation and completely characterize the toxicity
differentials of the agents. The direct estimate of the precision of the TDI makes it
easy to apply standard statistical significance tests or confidence intervals. In short
then, the TDI is well suited to the job at hand, the selection of those agents which
are promising from the standpoint of toxicity differential.

The main advantage of the TDI over the usual indices stems from the fact that
several arbitrary assumptions or decisions are avoided. While a number of variants of
Ehrlich's original Chemotherapeutic Index are used they have certain common features.
The numerator involves the per cent tumor inhibition (and the dosage at which a given
per cent inhibition is reached). Hence two arbitrary decisions are involved. First,
there is a choice of the level of inhibition, say 50 per cent inhibition in terms of
the linear dimensions of tumor. Second, the control series is singled out since it
serves as the base line for measuring inhibition. The denominator of the index will be
related to an arbitrary level of host toxicity (and the corresponding dosage). From a
theoretical standpoint, we would prefer to avoid unnecessary assumptions or decisions
(and the need for justifying such decisions). From a technical standpoint the experi-
mental situation in an animal screen makes these decisions both critical and dubious.
Because of the variety of dosage response curves that may be encountered (see Chart 2)
a minor change in the decisions could produce more changes -- or even reversals -- in
the rankings of the agents. Worse yet, it is technically difficult to keep a screen in
quality control so that we must expect such things as shifts up or down and other dis-
turbances (see Chart 2). With this kind of inherent variability, fixing the levels of
host toxicity presents serious practical problems (which can largely be avoided with the

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Furthermore, when we single out the control series we throw away the relevant information from the other points in the trial. We also raise technical problems in conjunction with the estimation of the corresponding dosages in the numerator and the denominator and in the estimation of the precision of the resulting index. The technical complications are reflected at the operational level. The net effect then, is that the usual indices introduce a whole series of unnecessary complications at the conceptual, technical, and operational levels.

The relative simplicity of the TDI makes it of potential value in situations where ordinary dosage response experiments are not feasible -- for example in clinical trials. If, for example, adequate measures of tumor and host toxicity are available, and if they are linearly related, then in theory it would be possible to compare two agents without requiring fixed dosage protocols for the patients. Research along these lines should be increasingly important in the future as the emphasis in the chemotherapy program shifts from the discovery of agents with tumor toxicity to the search for agents with large toxicity differentials.

The advantage of the TDI over the other indices is limited in the sense that both types of indices would be subject to other important limitations. The choice of the actual indices of tumor and host toxicity will be arbitrary in either case. For example, the index of host toxicity employed here, weight change, is easy to criticize (though it is not so easy to suggest a better index).

Readers who are used to working with percentage inhibitions may find the following interpretation of the TDI helpful. Suppose the TDI of Agent 16 is twice that of Agent 3, what does this mean in terms of percentage inhibitions? Simply this, if we consider dosage levels of the two agents that give the same host toxicity then the percentage inhibition in terms of linear dimensions of tumor for Agent 16 will be twice that for Agent 3. Although this point is fairly evident intuitively a mathematical proof is given in Appendix II.

There is one technical limitation of the TDI that deserves special mention. If the dosage levels for an agent do not get high enough to produce much tumor or host toxicity then the graph will have only a short segment of a straight line extending from the control point. In this event the slope is poorly estimated and therefore large slope estimates may occur as a result of sampling variation and may mislead the investigator. This happened in one trial of Agent 9 in these data. The problem of such outlying observations was overlooked in the first analysis of the data and this single "wild" estimate doubled the length of the confidence intervals. The graph showed that a single point on the dosage response curve -- the control point -- was responsible for the very large slope estimate. This point was out of line with the others in the trial and also with the other control values. This outlier was excluded in the subsequent analysis presented here. However, this experience suggests that it would be worthwhile to require that the dosage range in any trial be such that the segment of a straight line extends well beyond the control region. If this difficulty is noticed while the experiment is in progress it will be preferable to repeat the trial using higher dosage levels.

SUMMARY

The need for better indices of toxicity differentials has long been recognized. An index is suggested here which has some important advantages -- conceptual, technical, and operational -- over the usual indices in the experimental situations where there is a linear relationship between tumor and host toxicity indices. The ability of this index to discriminate between the agents under test is shown by the results of an actual screening experiment. The procedure looks as if it might be fruitful in other animal and clinical chemotherapy studies but further research is needed.
Several technical statistical points will be discussed in this appendix. These questions come up in connection with the analysis of a variance table based on the regression estimates of the slopes. There are twenty agents and three independent slope estimates for each agent. Hence for each agent there is a direct estimate, with two degrees of freedom, of the variation between trials on the same agent. In Table II the estimates have been pooled to give the mean square of 0.000573 with 40 degrees of freedom. The between trial variation and the between agent variation shown in Table II are calculated by usual ANOVA methods. However, there is a question as to whether the pooling of the estimates is appropriate here.

The third line of Table II is not obtained by the usual ANOVA procedures. It was calculated by combining the regression estimates of the variance of the slope estimate. The number of degrees of freedom for such variance estimates is two less than the number of dosage levels. For the sixty regressions there are, on the average, about 4 degrees, of freedom per regression but, a given trial may have anywhere from 2-6 degrees of freedom.

It will be seen from Table II that the variation between trials is about 50 per cent larger than the variation within trials -- a difference that is statistically significant at the 5 per cent level. This confirms the impression that the between trial variability is a more appropriate experimental error for these studies than the within trial variability. We can also see from Table II that the variation between agents is statistically significant at the 1 per cent level (F = 5.05) -- which confirms that the agents are different with respect to toxicity differential.

To investigate the legitimacy of pooling estimates, the average of the three regression variance estimates for an agent was plotted against the average slope for the agent. This graph showed a wide scatter (the largest estimate being almost eight hundred times the smallest). While there was some suggestion of a relationship between the size of slope and the variability (the agent with the largest slope also had the largest average within variance) the relationship was not very close. A plot of the average within variance for an agent against the between variance also suggested some relationship but again the relationship was not very close. These graphs indicated that the experimental situation involved heterogeneity of variance of a complex type. For about half of the agents the between and the within variances were similar, while for the other half the between variance was considerably greater. Nevertheless, the standard pooling procedure appears adequate as a first approximation. Pooling tends to make the intervals for agents with small slopes a little longer than they should be and the intervals for agents with large slopes a little shorter than they should be. Unfortunately no simple adjustment is available. For readers who wish to pursue this point, Table I gives the slope estimates in each trial, the regression estimates of variance for the slope estimate, and the direct estimate of the between trial variance for each agent.
APPENDIX II

THE MATHEMATICAL DEFINITION OF THE TDI

Let \( x_{ij} \) be the \( j \)-th dosage level of the \( i \)-th agent. Let \( \theta(x_{ij}) \) be the true host toxicity at this level and \( \phi(x_{ij}) \) be the true tumor toxicity. Then the slope at a given level is the derivative:

\[
\frac{d\phi(x_{ij})}{d\theta(x_{ij})} = \beta_1(x_{ij}).
\]

When there is a linear relationship between the tumor and host toxicity \( \beta_1(x_{10}) = \beta_1(x_{i1}) = \beta_1 \) since the slope does not depend on the level, \( j \). We can then write

\[
\phi(x_{ij}) = \beta_1 \theta(x_{ij}) + \alpha_1
\]

and the parameter \( \alpha_1 \) can be eliminated by considering the control dosage \( x_{10} \) where

\[
\phi(x_{10}) = \beta_1 \theta(x_{10}) + \alpha_1
\]

and \( \phi(x_{10}) \) and \( \theta(x_{10}) \) are the same for all agents \( \phi(x_{10}) = \phi_0, \theta(x_{10}) = \theta_0 \).

Subtracting (3) from (2) gives

\[
\phi(x_{1j}) - \phi_0 = \beta_1 \left\{ \theta(x_{1j}) - \theta_0 \right\}
\]

Mathematical notation is sometimes helpful in clarifying relationships such as the one cited in the text between TDI's and percentage inhibitions. For example the percentage inhibition for the \( i \)-th agent is:

\[
\text{PI}_i = 100 \frac{\phi(x_{ij}) - \phi_0}{\phi_0} = \beta_1 \left\{ \frac{\theta(x_{1j}) - \theta_0}{\phi_0} \right\}
\]

When the dosages of two agents give the same host toxicity, \( \theta(x_{1j}) = \theta(x_{2j}) \), then the quantity in brackets in (5) is the same for both agents and cancels out of the ratio:

\[
\frac{\text{PI}_1}{\text{PI}_2} = \frac{\beta_1}{\beta_2}
\]

The TDI is, in the linear case, the ratio of the slope for an agent to the slope for a reference agent (food restriction).

\[
\text{TDI}_i = \frac{\beta_1}{\beta_0}
\]

so that the ratio of the TDI's is the same as the ratio of the percentage inhibitions.

For the non-linear case the TDI can be defined as:

\[
\text{TDI} = \frac{\frac{d\phi(x_{1j})}{d\theta(x_{1j})}}{\frac{d\phi(x_{0j})}{d\theta(x_{0j})}} \bigg| \theta(x_{1j}) = \theta(x_{0j})
\]

but the procedure of this paper is no longer applicable.
ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. David Rodbard for his valuable help in the execution of computations and in the preparation of charts for this paper.

REFERENCES


### Table I

<table>
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### Table II

#### Analysis of Variance

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</table>

\[
F = \frac{0.002893}{0.000573} = 5.05
\]
**A New Approach to Differential Toxicity**

Irwin D. J. Bross and George S. Tarnowski


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