Use of Structure-Activity Relationships in Setting Priorities for Toxicologic Testing after Dermal Exposure

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BACKGROUND

Although great advances have been made in the last few years in the knowledge and use of structure-activity relationships (SAR) in toxicology, we are still a long way from being able to predict with confidence, the physiological activity of a substance using only knowledge of its chemical structure. On the other hand, toxicologists have been using SAR for years to make generalizations such as "polycyclic aromatic hydrocarbons are carcinogenic" and "chlorinated hydrocarbons are hepatotoxic" even with full recognition that there are many exceptions to such statements. In spite of the exceptions, these generalizations can be very useful in aiding in the design of a safety testing program. For example, if there is the possibility of significant exposure to a chlorinated hydrocarbon, certainly it would be necessary to look very closely at potential hepatotoxicity, designing the study so as to obtain the most useful data for risk assessment. In other words, SAR can be used even in such a broad sense when combined with exposure to set priorities for testing and to aid in designing the necessary studies.

A method reported previously (Cramer, 1978) uses SAR in combination with exposure in a systematic and rational manner using data on physiological activity of structural types, metabolic mechanisms, chemical reactivity and other relevant information to set priorities for testing of structurally defined, orally ingested organic and metallo-organic compounds. This method uses a "decision tree" of 33 questions, each answered "yes" or "no" and each leading either to another question or to a final classification into one of three classes (I, II and III) reflecting a presumption of low, moderate or serious toxicity. This classification when combined with exposure allows the setting of priorities for testing. Thus, a substance in class II with high exposure would have the highest priority for extensive toxicological evaluation. Conversely, a class I substance with low exposure would have very low priority for study and only minimal data would be required.

Three questions from the decision tree for orally ingested substances have been picked as examples to illustrate the method and serve as an introduction to the discussion as to how this method may be used in situations involving dermal exposure.

Question number 2 asks:

Does the substance contain any of the following functional groups: an aliphatic
(A) secondary amine or a salt thereof, cyano, N-nitroso, diazo (e.g. CH₂N₂), triazeno (RN=HNH₂) or quaternary nitrogen, except in any of the following forms: >C=N+R₂, >C=N+H₂ or the hydrochloride or sulphate salt of a primary or tertiary amine?

This question puts into class II some substances that contain elements, valency states, or functional groups often associated with significant toxicity. Such substances include nitrobenzenes and N-nitroso compounds. (The “A” in the second line refers to the definition of aliphatics as olefinic and polyolefinic but not acetylenic or alicyclic compounds.)

Question number 5 asks:

Is it a simply branched (I) acyclic aliphatic (A) hydrocarbon or a common carbohydrate?

This question puts the generally innocuous hydrocarbons and carbohydrates into class I, the presumptively least toxic class. (The “I” refers to the definition of simply branched as, “branched at C-C bonds, with branches of two or more C atoms at not more than two points along the main chain, with no secondary branching. Multiple branching, consisting only of 1-carbon moieties, falls within this definition.”)

Question number 6 asks:

Is the substance a benzene derivative bearing substituents consisting only of (a) hydrocarbon chains or 1′-hydroxy or hydroxy ester-substituted hydrocarbon chains and (b) one or more alkoxy groups, one of which must be para to the hydrocarbon chain in (a)?

This is a more complex question designed to deal with certain substances related to safrole, some of which have been shown to have carcinogenic activity. Such substances include estragole but not the closely related eugenol (Miller, et al., 1983). The metabolic justification for separating these closely related materials is given below. A “yes” answer to this question results in a class II while a “no” answer leads to further questions.

A large number of pesticides, drugs, food additives and industrial and environmental chemicals of known biological properties were put through the decision tree. The results were that no class I substance had a no-effect level less than 50 mg/kg/day and no class II substance had a no-effect level of less than 5 mg/kg/day. There is no lower limit for class III substances but another order of magnitude lower in no-effect level, i.e. 0.5 mg/kg/day, encompasses all but the most severely toxic materials. Thus, one can use these lower limits of each class combined with an exposure value to calculate a “protection index”. For example, if the daily exposure to a chemical is in the range of 0.1 to 1 mg and it is a class I substance, the protection index would be 50 mg/kg (the lower limit for class I) × 50 kg (body weight)/mg (high end of range of exposure) = 2,500. The actual safety factor will be substantially higher than this figure due to the conservative assumptions that the substance is the most toxic in its class and has the highest exposure in the range.

Thus, the classification of presumed toxicity combined with ranges of exposure combined as shown in Table 1 allows an assignment of priority based on an estimate of the protection index calculated assuming the highest exposure and highest toxicity for each grid position. Four categories of priorities were set and designated A–D. Priority “A” represents the lowest priority and the requirement of only minimal data. Priorities “B” and “C” represent progressively higher priorities calling for more extensive data and priority D implies the highest priority for extensive toxicological evaluation including, in most cases, chronic studies.
MODIFICATIONS FOR DERMAL EXPOSURE

The above discussion illustrates how the decision tree method works for orally ingested substances. It should be possible to develop a similar system or to modify this system for substances exposed by the dermal route. Some modifications are obvious; others are more troublesome.

The "oral decision tree" has two questions related to the assumption that simple esters are readily hydrolyzed in the GI tract prior to absorption. This is obviously not an assumption that can be made for dermal absorption even though hydrolysis can occur as a substance passes through the skin.

There is also a question in the "oral decision tree" that allows a substance with fairly innocuous functional groups but nevertheless in class III to be raised to class II if it is a common component of food, that is occurring in a significant quantity in at least one major food or at lower levels in a wide variety of foods. This is based on the assumption that the decision tree has been overly conservative for these substances as witnessed by the fact that foods containing such substances are commonly eaten without harmful effects. This again is not an assumption that is applicable to dermal exposure.

Removal of these three questions results in a more severe classification, however, there is another consideration that often works in the opposite direction, that is the degree of skin absorption. If, for example, less than 1% of the substance is absorbed, this would be equivalent to lowering the systemic exposure by two orders of magnitude resulting in a lower priority class by the scheme in Table 1. Unfortunately, skin absorption data are not available for most substances, thus, it is necessary to either (a) assume in the absence of evidence to the contrary that the substance is completely absorbed, (b) develop a SAR scheme for skin absorption or (c) actually measure the absorption. At this time not enough data are available to take advantage of option "b" the most desirable option. Option "a" will be too conservative for many substances and thus option "c" must be invoked. This does not mean, however, that skin absorption studies must be done on all substances exposed dermally, but only for those in the higher classes of priority.

Of course, one other major difference between dermal and oral exposure is the possibility in the former of topical effects such as contact
sensitization or phototoxicity. Here a SAR scheme is being developed. In the meantime, it is necessary to conduct screening tests for these effects on all substances for which significant dermal exposure is expected.

**METABOLIC CONSIDERATIONS**

The principle basis for the decision tree approach and, indeed, for all SAR schemes is that similar chemical structures have similar chemical reactivity, are metabolized similarly, and, therefore, have similar physiological activities. While there is good evidence in numerous cases for this generalization, it must be used with caution because knowledge of metabolic pathways is poor or nonexistent for many chemical types and for many more is insufficient to explain subtleties of observed variations. This is the reason that it is sometimes necessary to conservatively overclassify substances, that is, to place them in a presumptively more toxic class simply because knowledge of metabolic pathways is insufficient to distinguish among structures that are grossly similar but may have small differences that result in greatly different physiological activities. For example, bay region theory notwithstanding, knowledge of metabolic activation of polycyclic aromatic hydrocarbons (PAH) is still insufficient to place with confidence such a structure in the presumptively least toxic class even though on testing it may show no carcinogenic activity. It must be kept in mind that this is a method for setting priorities not for predicting toxicity and such a conservative classification is only saying that if there is significant exposure to an untested PAH that substance would have a high priority for testing.

On the other hand, it is possible to make relatively fine distinctions when knowledge of metabolism and chemical principles is sufficient to allow such distinctions to be made with confidence. Two examples have been picked to illustrate this point.

First, let us look at question 5 as mentioned above. This question was designed based among other things on the generalization that no simple acyclic hydrocarbons had been shown to have significant toxicity. This was prior to the time that neurotoxicity of hexacarbon compounds including hexane became well known (Spencer, 1980). Even though, the no effect level for such substances is apparently greater than 50 mg/kg/day which would argue for a class I, knowledge of the mechanism of the neurotoxicity is insufficient to place all aliphatic hexacarbons in class I. This question, therefore, should be modified to route such substances into class II. Such a classification would result in a high priority for subchronic testing for any exposure greater than 0.01 mg/day.

A second example is illustrated by question 6. As mentioned above, this question separates certain substituted benzene compounds related to safrole from others that have similar structures yet have not shown similar activity. The two substances in these categories that have been studied most are safrole and eugenol (see **Table 1**). Safrole is classified as a carcinogen in rats and mice (HHS, 1982) while eugenol was not shown to be carcinogenic by the NTP after a 2 year bioassay in rats and mice (NTP, 1982). The proximate carcinogen of safrole has been shown to be the 1'-hydroxy metabolite (Bochert, 1973; Drinkwater, 1976; Wislocki, 1977) and although this metabolite was not detected in man (Benedetti, 1977) the FDA has banned the food use of safrole (41 FR 19207, 1976). More recently the Millers have shown that the ultimate carcinogenic metabolite
in mice is the sulfuric acid ester of 1′-hydroxysafrole (Miller, 1983). This is completely consistent with the theory that highly electrophilic substances can be genotoxic carcinogens. It can then be asked why eugenol with a very similar structure is not metabolized to a similar electrophilic species. There is one major difference in the two substances. Eugenol has a free phenol group allowing as a ready metabolic pathway, conjugation and excretion. Such a pathway is possible with safrole only after breaking one of the carbon oxygen bonds of the methylene dioxy group, a relatively slow reaction. Likewise, oxidation at the 1′-position

**Table 2**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Class</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFROLE</td>
<td>I1</td>
<td>Carcinogenic to rats and mice (HHS, 1982) Positive in many mutagenicity screens</td>
</tr>
<tr>
<td>ISOSAFROLE</td>
<td>I1</td>
<td>Weakly carcinogenic to mice (Miller, 1982) Negative in many mutagenicity screens</td>
</tr>
<tr>
<td>DIHYDROSAFROLE</td>
<td>I1</td>
<td>Weakly carcinogenic in mice (Miller, 1982) Negative in Ames test (Wislocki, 1977)</td>
</tr>
<tr>
<td>ESTRAGOLE</td>
<td>I1</td>
<td>Weakly carcinogenic in mice (Drinkwater, 1976) Negative in Ames test (To, 1982)</td>
</tr>
</tbody>
</table>
is a relatively slow reaction and thus competes effectively when no more facile pathway is available. In other words, where a free phenol group is present, conjugation and excretion is sufficiently rapid that 1'-oxidation does not have time to occur to a significant degree.

Work is now underway to confirm this theory. Another part of this question refers to the necessity of alkoxy groups one of which must be para to the alkyl side chain. This distinction refers to the known activating effect of such a group by stabilization of the incipient
carbonium ion that would be the electrophilic species of the 1'-sulfoxy ester metabolite.

Table 2 compares the decision tree classification for several substances with the reported carcinogenic or mutagenic activity. This shows very good correlation with the above theory. That is, where there is the possibility of an activated methylene but no metabolic handle that would allow ready alternate metabolism, the substance should be considered high priority for at least mutagenicity screening if exposure is significant.

**UTILIZATION**

It cannot be emphasized too strongly that this method is to be used only for setting priorities and assisting in determining a reasonable level of testing. It is not a method for predicting toxicity. A classification of II does not mean a substance poses a risk, only that if such a substance has a high exposure it should be in the highest priority for evaluation. This method should be used in conjunction with other knowledge such as data from skin absorption studies, data on related materials, data from short-term screening studies, etc.

Figure 1 illustrates a systematic method for incorporating this method into an overall scheme of safety evaluation. Two examples will serve to show how the scheme works.

First, looking at a substance classified as priority "D" from the decision tree. This means some combination of SAR and exposure has resulted in high priority for evaluation. At this point skin absorption data and data from a basic battery of tests are required. Such a basic battery may include a dermal LD50, skin irritation data, mutagenicity screening, etc. If skin absorption is significant (for this example the figure of >5% is picked) a choice must be made between conducting extensive toxicological testing or restricting...
use such that the priority class is lowered. On the other hand, the priority class may be lowered by showing a low skin absorption resulting in a lower systemic exposure. In either case, it might be possible to then demonstrate with only subchronic testing a reasonable expectation of no harm under conditions of use. Of course, if data at that point raise questions that remain unanswered, it may be necessary to conduct additional testing.

On the other hand, a classification of A would, in the absence of unanswered questions, raised, for example, from customer or worker complaints or data on related materials, result in a very low priority for further testing at this time. It is possible, however, for a substance initially classified as "A" priority to proceed through the scheme all the way to extensive toxicological evaluation.

This is a preliminary presentation of a scheme for setting priorities for the testing of a substance to which the exposure is dermal. Of course, many questions remain to be answered such as: What further modifications of the decision tree are necessary? What kind of SAR is possible for topical effects such as are contact sensitization, phototoxicity, etc? Is it possible to develop SAR for skin absorption or in vitro methods sufficient for this scheme? It will also be necessary to test the method thoroughly to assure that it is acceptable. It should be emphasized that this is both an interactive and a dynamic process subject to changes as our knowledge of SAR, toxicology, and metabolism advances.

BIBLIOGRAPHY


[Discussions]

Tejima, M. (Shiseido): I am very much interested in the decision tree you presented here today and in its application in dermal toxicity testing. From an organic chemist's viewpoint, what structures or partial structures do you know to be associated with contact allergy?

Ford: There are several examples that can be given. One class of substances—the hydroxy-methyl substituted tertiary butyl phenols—was talked about by Dr. Malten. And another well known class is the unsaturated carbonyl compounds, especially the aldehydes. In general, materials that have a high nucleophilic reactivity would be high in priority both from a toxicological viewpoint and from a dermal toxicology viewpoint. The problem is complex because much of the data in the literature are difficult to interpret.
構造活性相関の応用
—皮膚接触物質の毒性試験の優先順位の決定—

Ford, R. A.

キーワード：構造活性相関（SAR）—decision tree—toxicology—priorities 優先順位

構造活性相関（SAR）に関する知識の進歩は近年著しいが、化学物質の構造からその生理活性を十分推定しうるとは至っていない。しかしどのような構造の物質の毒性試験を優先すべきかという安全性試験計画の立案上、SARは有用な手段となる。

経口摂取化学物質について、優先順位を決定する為に33の質問からなる"decision tree"が作られ、推定される毒性の程度からclass I～IIIの3段階に物質を分類する方法が開発された。この分類と曝露の程度からA～Dの4つのカテゴリーが選定される。Aの優先順は低く、最小限の資料のみが要求される。他方Dでは、慢性毒性試験を含めた広範囲な毒性評価が要求される。

この方法を皮膚に曝露される物質に適用するには、いくつかの問題点がある。単純エステルは吸収される前に消化管で加水分解を受けまるため、設定された質問と一し、明らかに無害な官能基より構成される物質でも、食物中で多く含まれる場合は優先順位が高くなることなどがその1例である。さらに考慮すべき点は、経皮吸収の問題で、優先順位の高い物質は、経皮吸収実験を行うことから望まれる。"Decision tree"で高い優先順位をあたえた物質は、経皮吸収、LD50、皮膚刺激試験、変異性試験などの毒性評価がなされる。

本法は優先順位の決定あるいは、適当な試験の範囲の設定上有用であり、多くの不十分な点はあるが、今後、SAR、中毒学、代謝の知識の進歩とともに改善されるであろう。

【討論】

手島正行（質生堂）：私たちは企業の安全性試験を担当する者にとっては、Dr. Fordの御紹介されたdecision-making treeの考え方は非常に興味深いものであります。特にDr. Fordは有機化学者でいらっしゃいますので、接触アレルギー物質の構造について何か有機化学的な観点からみた場合のコメントをございませんでしょうか。

フォード：特によく知られている化学構造としては
①Malteneがお話しになったphenol基を有する化合物。
②Aldehyde基を有する化合物。
③親核反応を起こすような化合物は強い接触アレルギーを誘発しうておる。しかしこういった問題は複雑で難しい。