Assessment of Carcinogenic Hazard of Chemical Mixtures through Analysis of Binary Chemical Interaction Data

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Assessment of the potential health hazard of environmental complex chemical mixtures is one of the most difficult and challenging problems in toxicology. In this article, we describe the development of an innovative computerized system for ranking and predicting potential cancer hazard of chemical mixtures. We take into consideration both the additive risk of individual carcinogens present and the projected overall interaction effect of the mixture based on analyzing and integrating the possible interaction effects of all binary pairs of individual constituents of the mixture. Using this system, it can be predicted that a number of mixtures of polycyclic aromatic hydrocarbons should have a carcinogenic risk lower than that calculated by the simple additivity model, whereas the reverse is true for a number of other mixtures. The system can be very useful in hazard ranking and priority setting in dealing with mixture problems such as cleanup of hazardous waste.—Environ Health Perspect 102(Suppl 9):113–119 (1994)

Key words: chemical mixtures, cancer hazard/risk, computerized system, interaction, synergism, antagonism, promotion, inhibition

Introduction

Assessment of the potential health hazard of environmental complex chemical mixtures is a difficult and challenging toxicological problem and a subject of major current concern to both the scientific and regulatory communities (1,2). This is particularly true when dealing with cancer as the toxicological end point. Besides the usual problems associated with risk assessment of individual chemicals, three major obstacles associated with mixtures are a) the impossibility of testing myriads of possible combinations of chemicals; b) the lack of a universally accepted index for quantitative measurement of cancer risk of chemical carcinogens; and c) the uncertainty of the possible outcomes of interactions among the various constituents of the mixture.

Despite this uncertainty, there is clear realization that the toxicologic evaluation of complex chemical mixtures is becoming increasingly important for the hazard assessment of environmental sites (3). The epitome of these unanswerable uncertainty are the problems of assessing the relative hazard that hazardous wastes disposal sites represent (4) and assessing the priority for cleanup and decontamination. Beyond this acute example, the problem also surfaces in connection with hazards represented by industrial effluents, pollutants in water and air, industrial products consisting of mixtures of chemicals, as well as complex human and veterinary medicinal preparations.

During the past six years, the U.S. Environmental Protection Agency (EPA) and the National Cancer Institute (NCI) have been involved in systematically compiling databases on combined effects of binary mixtures of carcinogens (5), carcinogens and inhibitors (6), and carcinogens and promoters (7). These databases provide crucial information needed for mixture assessment. In this paper, we describe an innovative and pragmatic approach for integrating the information extracted from these databases. We also describe the mathematical, statistical and toxicological considerations required to develop a computerized system, the Integral Search System (ISS). This system is capable of ranking and predicting the potential cancer hazard of complex chemical mixtures.

Conceptual Principles Used in Development of ISS

The principles and assumptions used in the development of ISS are described in the User's Manual that accompanies the software (8). Essentially, ISS consists of two major components: a component to calculate the "inherent (cancer) hazard" of the mixture based on an additivity model, and a "hazard modification" component to modify the "inherent hazard" by a "weighting ratio" calculated by analyzing and integrating the possible interaction effects of all the binary pairs of individual constituents of the mixture.

Inherent Cancer Hazard Component. The calculation of the "inherent hazard" using the basic additivity model is based on the assumptions that the concentrations of various chemical carcinogens present in the environmental mixtures are usually relatively low with respect to the dose–response curve, and the effects of constituents of a chemical mixture do not interact with each other. Under these conditions, the individual effects of various constituents present in the mixture can be combined in an arithmetically additive manner to provide an estimate of the overall effect. Owing to the lack of systematic method to correct for interaction effects, this basic additivity model has been virtu-
ally the only method used for risk assessment of chemical mixtures (9). Ideally, the additive effect should be calculated by taking the concentration of each chemical constituent into consideration (i.e., overall effect = S concentration × potency). However, realistically, for virtually all environmental mixtures, information on concentrations of constituents is either incomplete or nonexistent. Thus, for ISS, the overall effect is calculated by summing the potencies of individual constituents (i.e., overall effect = Σ potency).

There is no consensus on a universally accepted index for the potency of carcinogens. In ISS, for carcinogens with sound dose–response data, the slope factor* * (q₁*) has been used as an index of potency. For carcinogens with no q₁* values and for chemicals strongly suspected to be carcinogens, a structure–activity relationship (SAR) analysis may be used to provide a rough estimate of their potency. The use of SAR analysis allows inclusion of virtually any type of compound, thereby giving a more realistic estimate of the inherent hazard. Besides SAR, other alternative indexes or scales (e.g., TD₅₀, RfD) may also be used when sufficient data are available.

To allow conversion between the q₁* values and concern level scales, a correspondence table (Table 1) has been developed based on analysis of 134 carcinogens with q₁* values to reach a reasonably even distribution among narrative concern level scales. The conversion enables calculation of the arithmetic sum of the potencies of all carcinogenic constituents to give the inherent hazard of the mixture. Since interaction often modifies hazard by orders of magnitude, the inherent hazard must be converted to a linearized scale (exponent index) before application of the weighting ratio (WR).

### Table 1. Correspondence between the scale of slope factors, exponent indexes and concern level terms.

| Slope factor (q₁*) | Exponent index | Concern level |
|--------------------|----------------|---------------|
| 0 to < 5 x 10⁻⁶  | 0 to < 1       | Low           |
| 5 x 10⁻⁶ to < 5 x 10⁻⁵ | 1 to < 4     | Marginal      |
| 5 x 10⁻⁵ to < 5 x 10⁻⁴ | 4 to < 6     | Low-moderate  |
| 5 x 10⁻⁴ to < 5 x 10⁻³ | 6 to < 8     | Moderate      |
| 5 x 10⁻³ to < 5 x 10⁻² | 8 to < 10    | High-moderate |
| 5 x 10⁻² to 5 x 10⁻¹ | 10 to > 14   | High          |

*The Slope Factor (q₁*) is the slope of the straight line from the upper bound risk at zero dose producing an upper bound risk of 1%. It expresses the cancer risk (proportion of population affected) per unit of dose (in mg/kg/day or mmole/kg/day) (10). q₁* can be calculated using commercially available software, such as Global 86. The q₁* values of a number of carcinogens are available in on-line database such as U.S. EPA’s IIRIS database (11).

ISS is its capability to project a realistic estimate of the overall interaction effect of the mixture based on available interaction data and chemical class assignments. There are four standard response categories (SRCs) for interactions among chemicals that may cause deviation from the basic additivity model: synergism (syn), promotion (pro), antagonism (ant), and inhibition (inh). The former two represent hazard-amplifying interactions while the latter two represent hazard-reducing interactions. Depending on the relative balance of the totality of hazard-amplifying and hazard-reducing interaction effects, the inherent hazard level may be modified upward or downward. Two important and reasonable assumptions have been made. First, the overall interaction effect can be calculated by integrating the individual interaction effects of all the binary pairs of various constituents of the mixture. Second, in the absence of interaction data on any given binary pair of chemicals, the interaction of the binary pair can be approximated by the predominant interaction effect associated with the binary pair of chemical classes to which each chemical belongs.

The hazard modification component consists of five segments: (a) a computer program to generate all possible binary pairs of chemical constituents of mixture, (b) a master database integrating the three U.S. EPA/NCI databases on binary interaction of carcinogen with carcinogen/promoter/inhibitor, (c) search capabilities to locate interaction “hits” (Hₐ) for each binary pair of chemicals, (d) mathematical formalism to calculate the adjusted “inferred” values (Hᵦ) for each pair of chemicals for which the interaction cannot be located during the search but can be inferred by association to a pair of structural or functional classes to which the chemicals belong, and (e) mathematical formalism to calculate the weighting ratio.

**Segment A.** The number (N) of combinations (binary, ternary, etc.) that can occur between the number of individual chemicals present in a mixture is given by the factorial formula:

\[ N = \frac{(n - 0)(n - 1)(n - 2) \ldots (n - (k - 1))}{k!} \]

where \( k \) is the multiplicity of the combination. For binary mixtures (\( k = 2 \)), it can be shown that the number of binary pairings increases rapidly with the number of chemicals (e.g., \( N = 45 \) for 10 chemicals; \( N = 4950 \) for 100 chemicals). Owing to the speed of computers, it is feasible to establish binary pairing between any number \( n \) of identified compounds in a mixture. ISS contains a program to generate such a set of binary pairs to search for interaction “hits” in Segment C.

**Segment B.** The ISS master database, integrating and standardizing the three U.S. EPA/NCI databases on binary inter-

The interaction effects involving chemical carcinogens fall into three types: (A) When both chemicals are established carcinogens, the interaction effects may be: (i) additivity, when the combined effect is additive, (ii) synergism, when the combined effect is less than additive, and (iii) antagonism, when the combined effect is less than additive. (B) Inhibition, when exposure is to an established carcinogen at an effective dose, together with or preceded or followed by exposure to a non-carcinogenic chemical agent that is an inhibitor of the carcinogenic response. (C) Promotion/cocarcinogenesis, when exposure is to an established carcinogen at subcarcinogenic levels or to a very weak carcinogen, and this exposure is followed by (in the case of promoter) or simultaneous with (in the case of cocarcinogens) exposure to a noncarcinogenic chemical agent that significantly enhances the carcinogenic response.
action of carcinogen with carcinogen/promoter/ inhibitor, contains carcinogenesis
binary combination effects about 1000
chemicals of 60 structural/functional
classes. It is structured to allow searching
by specifying binary pairs of individual
chemical names, as well as binary pairs of
chemical class terms.

Segment C. The ISS search program is
designed to search for matching pairs
between the set of binary pairs generated in
Segment A and those present in the master
database to locate interaction "hits" of the
four SRCs that may cause deviation from
the basic additivity model. For each binary
pair of individual chemicals, a "name pair
hit" is registered if interaction data on this
specific pair is located in the master
database. The number of name pair hits of
the mixture is then tallied to give the total
number \(N_s\) for each of the four SRCs.

Segment D. For each binary pair with
no name pair hit, ISS continues to search
for interaction hits associated with the
binary pair of structural/functional classes
(class pair hits) to which the chemicals
belong. Since class pair hits only represent
possible interaction based on class associa-
tion, they are given less weight than name
pair hits. For each SRC, the raw score of
the total number of class pair hits is tallied
and then statistically adjusted to give the
class pair inferred value \(H_s\). The statisti-
cal adjustment of raw score of class pair
hits involves consideration of the number
of documented interactions between each
class pair in the ISS database, the distribu-
tion of the type of SRC effect within a class
interaction, and the representativeness of
classes in the ISS database (8). The equa-
tion derived for these adjustments is:

\[
H_{s \text{ effect }} = \left[ \frac{(O_{\text{effect}})^2}{E_{\text{effect}}} \right]_{\text{Lim} \rightarrow 1}
\]

Where:

- \(O_{\text{effect}}\) = the number of com-
  pounds in class x.
- \(E_{\text{effect}}\) = the number of com-
  pounds in class y.

The maximum limiting value of 1 has
been placed on each \(H_{s \text{ effect }}\) because
the contribution of a class pair interaction
calculated inferred value should not exceed
that of a name pair hit.

For each SRC, the total interaction
effect of the mixture with respect to that
specific SRC is the sum of \(H_A\) and \(H_B\):

\[
H_{\text{syn}} = H_A + H_B
\]

Segment E. The extent of hazard mod-
ification due to interaction is dependent
on relative balance between hazard-amplifying
and hazard-reducing interactions. This can
be best represented by the general form:

\[
WR = \frac{\text{Hazard-Amplifying Interaction Effects}}{\text{Hazard-Reducing Interaction Effects}}
\]

The algebraic form of the weighting
ratio is given by the equation:

\[
WR = \frac{1 + (p H_{\text{syn}} + q H_{\text{pro}})}{1 + (r H_{\text{ant}} + s H_{\text{inh}})}
\]

where the \(p, q, r,\) and \(s\) are hazard-modification
effectiveness coefficients which
reflect the effectiveness of the four types
of combination effects to modify the inherent
hazard level of chemicals. These coeffi-
cients are empirical and should reflect the
user's perspective of the entire combination
effects literature as well as conceptual
biases. It is the present view of the authors
that \(p = 0.3, q = 0.7, r = 0.3\) and \(s = 0.6\) are
reasonable values. However, it is only
through extended testing of this ranking
system and additional experimental data
becoming available in the future that an
increasingly accurate set of values for these
coefficients will be reached.

The presence of the unit number in
both the numerator and the denominator
of the WR provides an equation that will
yield a working and realistic WR value
even in limit circumstances when the haz-
ard amplifying/reducing ratio would
become zero or infinity either as a result of
actual data or because of partial absence
of data. Furthermore, this equation will yield
WR = 1, and will leave the inherent hazard
invariant when the hazard amplifying/
reducing ratio is 1 because of complete
balance of hazard amplifying/reducing effects,
and steps for search for hits in the ISS master database and for computation of a weighting ratio for adjusting the inherent hazard.

Once the list of chemical names of the compounds in a mixture is established and the slope factors or concern level terms are introduced, the program computes the inherent hazard. The compounds are then paired into all of the possible binary combinations of names. The ISS uses these name pairs to search for matching pairs present in the database. If a specific name pair is found in the database, that specific interaction (or interactions) is counted as a name hit and the search for that name pair is terminated.

Since not all possible chemical name pairs can be found in the ISS database, it is most probable that no interaction hit will be registered for many of the name pairs. Each of these chemical name pairs is then converted into its chemical class pair, so that a class interaction search can be carried out. If any interaction exists between the two classes in a pair, the extent of interaction is calculated and that inferred value is regarded as a class pair hit; the search for that class pair is then terminated.

As a safeguard against the possibility that some chemicals may have been missed (in a search by chemical class search criterion), because of improper or incomplete class term assignment (CTA), the system provides a listing of those chemical classes that did not intersect with any binary pairing of classes in the three databases ("no hit" classes). The chemicals in the no hit classes are identified and rerouted for "Criteria Review and Adequacy Control of Chemical Structural Class Assignment" to verify the correctness of the CTA, and the search may be repeated using the new CTA (if any).

**Application to Sample Mixtures**

To illustrate the use and the reasonable operation of the ISS system, four sample mixtures have been tested using the system. Table 2 summarizes the results of the ISS-generated hazard modification weighting ratios (WR) for these mixtures. Mixture 1, a polynuclear aromatic hydrocarbon (PAH) mixture containing two potent carcinogens (benzo[α]pyrene and 7,12-dimethylbenz[a]anthracene) and three weak or inactive compounds, has a WR of 0.63 indicating that the combined effect is expected to be less than that calculated by using an additive model. This is consistent with data review that the predominant interaction among PAHs is antagonism (15) and the experimental findings (16) that several PAH mixtures tend to have lower carcinogenic potential than that expected by adding the carcinogenic potentials of individual PAHs. Not all PAH mixtures are expected to interact identically. This can in fact be projected by the ISS system. A slight change in the chemical composition of mixture 1 (replace 3,9

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### Table 2. ISS-generated hazard modification weighting ratios of selected sample complex mixtures.

| Mixture | Constituent chemicals in mixture | Weighting ratio |
|---------|----------------------------------|-----------------|
| 1       | Benz[a]anthracene; benzo[a]pyrene; 7,12-dimethylbenz[a]anthracene and 3,9-dimethylbenz[a]anthracene | 0.63 |
| 2       | Benz[a]anthracene; benzo[a]pyrene; 7,12-dimethylbenz[a]anthracene and cyclopenta[c]pyrene | 0.61 |
| 3       | Benz[a]anthracene; benzo[a]pyrene and butylated hydroxytoluene (BHT); 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and diethylnitrosamine | 1.50 |
| 4       | Benz[a]anthracene; croton oil; phenol, pyrene and urethan | 2.52 |
dimethylbenz[a]anthracene with cyclopenta[cd]pyrene yields a mixture (mixture 2) with a higher WR of 0.81 reflecting a partial offsetting of the predominant antagonism among PAHs by the known synergistic interaction (17) between the specific pair of benz[a]pyrene and cyclopenta[cd]pyrene.

In contrast to the hazard-reducing interaction among PAHs, the WR of mixtures 3 and 4 exceeds 1.0, indicating that the combined effect is expected to be greater than that calculated by using an additivity model. The ISS-generated screen which shows the interaction profile that contributes to a WR of 1.5 for mixture 3 is shown in Figure 2. There are seven name pair hits—four hazard-enhancing (three promotion and one synergism), two hazard-reducing (two inhibition) and one neutral (i.e., additive)—and a variety of class pair hits. Using the formulae and statistical considerations previously described, these hits yield an overall WR of 1.5.

The ISS-generated WR for mixture 4 is 2.52 mainly because of multiple promotion hits with few or no hazard-reducing hits. The impact of applying this WR to the assessment of potential cancer hazard is depicted in Figure 3. There are no \( q_{1}^{*} \) values available on any of the compounds in mixture 4. Using SAR consideration, a concern level of low-moderate has been assigned to benz[a]anthracene and urethan. Croton oil is given a concern level of marginal. Using the correspondence table (Table 1), these concern levels can be converted to estimated \( q_{1}^{*} \) values (\( 1 \times 10^{0} \) for low moderate and \( 1 \times 10^{-2} \) for marginal) for the purpose of calculation of overall inherent hazard. Using the additivity model, the estimated overall inherent hazard \( q_{1}^{*} \) is calculated to be \( 2.01 \times 10^{0} \), which is still in the low-moderate range. After applying the WR of 2.52 to the exponent index, the final adjusted estimated overall cancer hazard \( q_{1}^{*} \) becomes \( 4.06 \times 10^{6} \), which is in the high-concern range. This example illustrates the importance of taking interaction effects into account in assessing the potential cancer hazard of mixtures.

**Conclusion**

In brief, we have developed an innovative computerized system capable of giving approximate but realistic assessments of the potential cancer hazards of chemical mixtures. The ISS is the first scientific attempt where all known interactions in chemical carcinogenesis are brought to bear on the cancer hazard assessment of complex chemical mixtures. The information generated by ISS is highly useful to both the scientific and regulatory communities for hazard ranking and for stimulating strategic research.

It is important to realize that no conceptual development of any hazard ranking procedure can remedy the considerable uncertainties that may surround the experimental data on which the ranking is based. Great approximations, tenuous extrapolations, imperfect and incomplete study designs, and enormous data gaps are sometimes encountered in the literature on the carcinogenic effects of chemical combinations. Critically lacking are studies on dose–response relationships in chemical interactions relative to carcinogenesis. Among the mass of data that have been analyzed and tabulated in the ISS master database, studies involving dose–response relationship of the interacting partners of chemicals are virtually nonexistent. It is recognized that the validity of ISS cancer hazard assessments can be limited by this dearth of data because the dose–response relationships can be of critical importance in some instances where, depending on experimental conditions and levels of the interacting compounds, the same combination may be hazard amplifying or hazard reducing (19). Furthermore, there is no standardization or accepted method of evaluating or expressing the intensity of the different combination responses. These uncertainties and limitations must be kept in mind when evaluating the carcinogenic effects of chemical combinations.

Aside from its capability to assess the potential cancer hazards of chemical mixtures, ISS is also a unique system in which all scientific data on binary interactions
involving chemical carcinogens can be centralized in one organized database. It is hoped that the development of ISS will stimulate more research to fill important data gaps and to design new experiments to challenge the conceptual framework and assumptions made in the ISS.

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