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Multifactor Potency Scheme for Comparing the Carcinogenic Activity of Chemicals

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A scheme for ranking the quantitative activity of chemical carcinogens is described. This activity scheme uses as its base dose potency measured as TD_{50} which after conversion into an inverse log scale, a decile scale, is adjusted by weighting factors that describe other parameters of carcinogenic activity. These factors include positive or negative weightings for the induction of tumors at tissues or organs associated with high historical control tumor incidences; the induction of tumors at multiple sites; the induction of tumors in both sexes of the species; and the induction of tumors in more than one species. These factors were chosen because they represented qualitative descriptions of the general specificity or nonspecificity of chemicals with regard to the activity in rodents and have some bearing on the potential activity of chemicals in humans. To construct a measure to express the inactivity of chemicals toward the induction of cancer, a measure analogous to the TD_{50} has been developed: highest average daily dose (HADD) in milligrams chemical/kilogram body weight administered in a chronic cancer study and that did not induce a statistical increase in tumors. HADD values were similarly converted to log decile units and adjusted by weighting factors according to lack of activity in both sexes of a species and the lack of activity in more than one species. Three activity ranking schemes were developed: the carcinogen activity–F344 rat, an activity scheme based on cancer data obtained with the F344 rat; the carcinogen activity–$B6C3F_1$ mouse, an activity scheme based on cancer data obtained with the $B6C3F_1$ mouse, and the carcinogen activity combined, an activity scheme based on selecting data from both the F344 rat and $B6C3F_1$ mouse.

Introduction

Several models for estimating the carcinogenic activity of chemicals have been proposed in which activity or potency is described in combinations of terms of administered dose, tumor incidence, or tumor latency (*1–4*). While each of these potency models is useful, there is a need to develop a cancer activity scheme that incorporates other factors in addition to dose in its construct. These factors should account for the specificity or nonspecificity of chemicals with respect to the ability to induce cancer in more than one sex of an experimental test species as well as the ability of the chemical to induce tumors in more than one test species. These characteristics are particularly important if the end point of concern is the potential of that chemical to induce cancer in man. Chemicals that are nonspecific with regard to tumor site, sex, and species of experimental animal are more likely to be a potential hazard to man. This is based on the fact that the majority of chemicals evaluated by the International Agency for Research on Cancer (IARC) and rated as sufficient evidence of carcinogenicity based on human data are also carcinogenic in experimental animals (*5*) and that most of these human carcinogens are also highly active as genetic toxins (*6*). Therefore, it was of interest to integrate dose–response relationships with factors that describe the generality or specificity of chemicals with regard to target species, sex, organs, and tissue sites. A complete description of this work is found in Nesnow (*7*).

Results

In this cancer-activity ranking scheme, the following factors were incorporated: an estimate of dose potency as the major determinant; a weighting for tumorigenic responses in organs or tissues that have a low historical control tumor incidence; a weighting for tumorigenic responses in multiple tissues or organs; a weighting for tumorigenic responses in both sexes of the same species; and a weighting for tumorigenic responses in multiple species.

Because dose potency would be the major determinant, the method for applying the weightings was to directly adjust the dose potency value after converting it to a log numeric scale. In applying these weightings, it was necessary to select a base increment adjustment value, and the concept of doubling dose was employed for this purpose (*8*). Therefore, weighting factors increasing or decreasing the dose potency value of chemicals by factors of 2 were established.

In constructing this activity scheme that compares the responses of different chemicals, it was desirable to compare data generated by administering chemicals by the same route to the same sexes, strain, and species of test animal. Comparisons made between the activity of chemicals would therefore be made

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using a common set of variables. It was also desirable to use data derived from standardized protocols, with standardized numbers of animals trreated, sufficient numbers of tissues examined, and appropriate quality-control procedures. Therefore, in construction of this scheme, the National Toxicology Program/National Cancer Institute (NTP/NCI) bioassay data (*9–11*) from diet, water, or gavage routes of administration to F344 rats and $B6C3F_1$ mice were used. This would allow the creation of two independent cancer activity rankings, one based on data generated in the F344 rat and one based on data generated in the $B6C3F_1$ mouse, and each based on one generalized route of administration.

For dose potency, the TD_{50} data were used (*4,12*). The selection of individual TD_{50} values found in Gold et al. (*12*) and earlier references for each sex-species combination of each chemical was predicated on three rules: *a*) If several TD_{50} values were available for a sex–species combination, then the one with the lowest value was chosen (the most sensitive end point). *b*) In the Gold et al. (*12*) reports, several TD_{50} values are calculated for each chemical. Only TD_{50} values with a probability of $p \leq 0.05$ were chosen. *c*) Only TD_{50} values were used for those studies rated as positive in the NTP/NCI technical reports (*9–11*).

For those chemicals found to be inactive in a specific sex–species the highest average daily dose (HADD) administered to the test combination, animals was used as a measure of inactivity. The HADD values in milligram chemical administered/kilogram body weight/day were calculated according to the methods described by Gold et al. (*12*) from the highest dose administered as described in the NTP/NCI Technical Reports (*9–11*). Chemicals that were tested by the NTP/NCI in the F344 rat and the $B6C3F_1$ mouse by oral routes of administration and that were found not to induce tumors in all four sex–species combinations were also included with their HADD values. In total, 142 chemicals were selected. Table 1 lists some examples of these chemicals.

A log method was applied to the TD_{50} values and HADD values to convert them into a numeric scale for comparisons among chemicals. These converted TD_{50} values are termed decile units and are inversely related to TD_{50} values by the formula:

$$\text{decile value (active chemical)} = \log[10^7/TD_{50}]$$

Decile values for active chemicals (carcinogens) are positive and range from >0 to 14.

A similar concept was used to convert the HADD values for the inactive chemicals (noncarcinogens) with the formula:

$$\text{decile value (inactive chemical)} = -\log[\text{abs(HADD)} \times 10^3]$$

Decile values for inactive chemicals are negative and range from <0 to -10. Decile values for individual sex–species comparisons for several of the 142 chemicals are found in Table 2.

Table 1. Examples of chemicals selected for comparison and their TD_{50} values (for carcinogens) or highest average daily dose (HADD) values (for noncarcinogens).[a]

Chemical name	CAS number	NTP/NCI Technical Report no.	TD_{50} or HADD, mg/kg body weight/day			
			F344 rat		$B6C3F_1$ mouse	
			Male	Female	Male	Female
Acetohexamide	968-81-0	50	-770	-963	-1526	-1653
Aldicarb	116-06-3	136	-0.24	-0.3	-0.72	-0.78
Allyl isothiocyanate	57-06-7	234	54.3	NA[b]	-17.9	-17.9
2-Aminoanthraquinone	117-79-3	144	101	-70.9	755	1490
3-Animo-4-ethoxyacetanilide	17026-81-2	112	-414	-518	2070	-845
3-Amino-9-ethylcarbazole HCl	132-32-1	93	28.1	55.1	46.4	33
1-Amino-2-methylanthraquinone	82-28-0	111	34.1	115	-53.6	NA
4-Amino-2-nitrophenol	119-34-6	94	309	NA	-294	-319
2-Amino-5-nitrothiazole	121-66-4	53	27.6	-29.7	-11.9	-12.8

[a]From Nesnow (*7*).
[b]NA, not applicable.

Table 2. Examples of the calculation of unadjusted and adjusted individual sex–species values for the male and female F344 rat and $B6C3F_1$ mouse in decile units.[a]

Chemical name	Individual sex–species value (decile units)							
	Unadjusted				Adjusted			
	F344 rat		$B6C3F_1$ mouse		F344 rat		$B6C3F_1$ mouse	
	Male	Female	Male	Female	Male	Female	Male	Female
Acetohexamide	-5.89	-5.98	-6.18	-6.22	-5.89	-5.98	-6.18	-6.22
Aldicarb	-2.38	-2.48	-2.86	-2.89	-2.38	-2.48	-2.86	-2.89
Allyl isothiocyanate	5.27	NA[b]	-4.25	-4.25	5.27	NA	-4.25	-4.25
2-Aminoanthraquinone	5.00	-4.85	4.12	3.83	5.00	-4.85	3.82[c]	4.43[d]
3-Amino-4-ethoxyacetanilide	-5.62	-5.71	3.68	-5.93	-5.62	-5.71	3.68	-5.93
3-Amino-9-ethylcarbazole HCl	5.55	5.26	5.33	5.48	6.45[d]	6.16[d]	5.03[c]	5.48
1-Amino-2-methylanthraquinone	5.47	4.94	-4.73	NA	6.37[d]	4.94	-4.73	NA
4-Amino-2-nitrophenol	4.51	NA	-5.47	-5.50	4.51	NA	-5.47	-5.50
2-Amino-5-nitrothiazole	5.56	-4.47	-4.08	-4.11	5.56	-4.47	-4.08	-4.11

[a]From Nesnow (*7*). Conversion of TD_{50} values (from Table 1) into decile units.
[b]NA, not applicable.
[c]These TD_{50} values in decile units were adjusted (-0.3 decile units) for TD_{50} values based solely on tumors with high historical control tumor incidences ($\geq$ 10%).
[d]These TD_{50} values in decile units were adjusted (+0.9 decile units) for tumors appearing at more than one organ or tissue site.

The multifactor carcinogen activity scheme was constructed to represent the activity of chemicals with respect to dose and to the induction of tumors at sites associated with low historical control tumor incidence, the induction of tumors in more than one organ or tissue site, the induction of tumors (or lack of induction of tumors) in both sexes of F344 rats and/or B6C3F$_1$ mice, and induction of tumors (or lack of induction of tumors) in both F344 rats and B6C3F$_1$ mice. The individual steps in this process are outlined in Figure 1. The procedures used at each step in the calculations are described below.

An adjustment to the decile values for each sex–species combination was made to account for the magnitude of the historical control tumor incidence values at the sites of the tumors used in the calculation of the TD_{50} value. A decrement of 0.3 decile units was applied to the TD_{50} value in decile units for each sex–species combination who TD_{50} value was based on tumors with an associated historical control tumor incidence of $\geq 10\%$ (*13*). This had the effect of doubling the TD_{50} value (Table 2).

Because chemicals that induce tumors at more than one organ or tissue site are of more concern, the decile values of those sex–species combinations exhibiting tumors at multiple organs or tissues were increased. This was accomplished by increasing the decile value by 0.9 decile units for those sex–species combinations that demonstrated a statistical increase in benign or malignant tumors at more than one organ or tissue site as described in the NTP/NCI technical reports (*9–11*) (Table 2). This adjustment had the effect of reducing the TD_{50} value by a factor of 8.

After calculating the four adjusted individual sex–species values, we calculated the carcinogen activity–F344 rat and the carcinogen activity–B6C3F$_1$ mouse values. For each chemical tested, the male and female adjusted individual sex–species values were compared to select the predominating adjusted individual sex–species value. This was performed for each species. Two rules were employed for the selection processes: a positive value took precedence over a negative value and a higher absolute numeric value took precedence over a lower absolute numeric value. These rules had the effect of selecting for the most sensitive (for tumorigenic activity) adjusted individual sex–species value for active chemicals and the least sensitive (for toxicity) sex–species combination for the inactive chemicals.

After selecting the predominating sex–species value for each species, a factor for activity (or inactivity) in both sexes (correspondence between sexes) was applied to give the carcinogen activity–F344 or the carcinogen activity–B6C3F$_1$ mouse, if the chemical was tested in both sexes of a species. A value of 0.06 decile units was added to the predominating adjusted individual sex–species value if both sexes had adjusted individual sex–species decile values that were positive (the chemical was active in both sexes). A value if −0.6 decile units was added to the predominating adjusted individual sex–species value if both sexes had adjusted individual sex–species decile values that were negative (the chemical was inactive in both sexes) (Table 3). This procedure raised the absolute numeric values of those chemicals that had been tested in both sexes and that gave corresponding results to yield the carcinogen activity–F344 rat or the carcinogen activity–B6C3F$_1$ mouse. A value of −0.54 decile units was added to the predominating adjusted individual sex–species value if both sexes had been tested and the adjusted individual sex–species decile values were positive and negative (noncorrespondence between sexes) (Table 3). The three factors used in

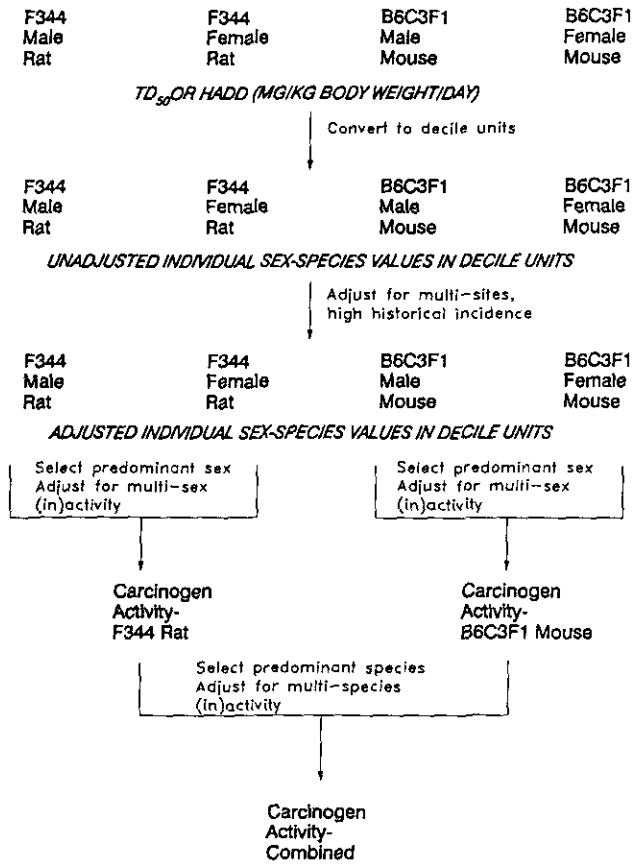

FIGURE 1. Steps taken in the calculation of the multifactor carcinogen activity scheme values for chemicals. From Nesnow (*7*).

these calculations were derived as follows: An overall factor of 0.6 decile units was selected as the base unit and was adjusted for the observed historical correspondence between the two sexes of each species to chemical carcinogens. The historical correspondences between the both sexes of F344 rat and the B6C3F$_1$ mouse are equivalent, 89.3%, based on a 222-chemical data set (*9*). Therefore, when the data are available for both sexes of F344 rats or B6C3F$_1$ mice, the factor that is applied to the results that are corresponding (both sexes positive) is 0.06 ($0.6 \times [1.0 - 0.893]$). The factor applied to the predominating adjusted individual sex–species value for results that are conflicting (one positive, one negative) is 0.54 (0.6×0.893).

The calculation of a combined species activity (carcinogen activity–combined) was performed in a similar fashion to the calculation of the carcinogen activity–F344 rat and the carcinogen activity–B6C3F$_1$ mouse. For each chemical, the carcinogen activity–F344 rat value and the carcinogen activity–B6C3F$_1$ mouse value were compared to select the predominating carcinogen activity species value using the following rules: a positive value took precedence over a negative value, a higher absolute numeric value took precedence over a lower absolute numeric value.

After selecting the predominating carcinogen activity species value for each chemical, a factor for activity (or inactivity) in both species was applied to give the carcinogen activity–combined. A value of 0.5 decile units was added to the pre-

Table 3. Examples of multifactor carcinogen-activity scale values for NTP/NCI chemicals.[a]

Chemical name	Carcinogenic activity		
	F344 rat	B6C3F$_1$ mouse	Combined
Acetohexamide	-6.58[b]	-6.82[b]	-8.32[c]
Aldicarb	-2.98[b]	-3.49[b]	-4.99[c]
Allyl isothiocyanate	5.27	-4.85[b]	4.27[d]
2-Aminoanthraquinone	4.46[e]	4.49[f]	4.99[g]
3-Amino-4-ethoxyacetanilide	-6.31[b]	3.15[e]	2.15[d]
3-Amino-9-ethylcarbazole HCl	6.52[f]	5.55[f]	7.01[g]
1-Amino-2-methylanthraquinone	6.43[f]	-4.73	5.43[d]
4-Amino-2-nitrophenol	4.51	-6.10[b]	3.51[d]
2-Amino-5-nitrothiazole	5.02[e]	-4.71[b]	4.02[d]

[a]From Nesnow (7).
[b]A factor of -0.60 decile units was added to the predominating adjusted individual sex-species value to give this number.
[c]A factor of -1.50 decile units was added to the predominating carcinogen activity-F344 rat or carcinogen activity-B6C3F$_1$ mouse value to give this number.
[d]A factor of -1.00 decile units was added to the predominating carcinogen activity-F344 rat or carcinogen activity-B6C3F$_1$ mouse value to give this number.
[e]A factor of -0.54 decile units was added to the predominating adjusted individual sex-species value to give this number.
[f]A factor of 0.06 decile uknits was added to the predominating adjusted individual sex-species value to give this number.
[g]A factor of 0.50 decile units was added to the predominating carcinogen activity-F344 rat or carcinogen activity-B6C3F$_1$ mouse value to give this number.

dominating carcinogen activity species decile value if both species had carcinogen activity decile values that were positive (the chemical was active in both species). A value of −1.5 decile units was added to the predominating carcinogen activity species decile value if both species had carcinogen activity decile values that were negative (the chemical was inactive in both species). A value of −1.0 decile units was added to the predominating carcinogen activity species decile value if both species had been tested and the carcinogen activity species decile values were positive and negative (noncorrespondence between species) (Table 3). This procedure raised the absolute numeric values of those chemicals that had been tested in both species and that gave corresponding results to yield the carcinogen activity-combined. The value of 1.5 decile value was selected as the base unit and was adjusted based on the observed historical correspondence between the F344 rat and the B6C3F$_1$ mouse (one or more sexes of each species were active and/or both sexes of both species were inactive), 66.7% (9). Therefore, when the data are available for both F344 rats and B6C3F$_1$ mice, the factor that is applied to results that are corresponding (both sexes positive) is 0.50 [1.5 × (1.0 - 0.667)]. The factor applied to the carcinogen activity species value for results that are conflicting (one positive, one negative) is 1.0 (1.5 × 0.667).

Discussion

The development of the multifactor carcinogen-activity scheme is based on a number of assumptions concerning the relative activity of chemicals. These include the general assumption that chemicals positive in more than one sex and more than one species should be considered more potent than chemicals that are not. In addition, chemicals that produce tumors in organs or tissues that are not associated with high historical control tumor incidences and chemicals that induce tumors at more than one organ or tissue site should also be considered more potent than those that do not. Based on these assumptions, the multifactor caracinogen-activity scheme has been developed, which incorporates all of these properties of a chemical into one unified basis for comparison. In this scheme, the overriding determinant of activity is dose. All of the previously described factors are used as adjustments to the original dose potency as determined for the active chemicals as TD_{50} or for the inactive chemicals as HADD. The maximum adjustment to dose potency for two chemicals (one that induces tumors at multiple sites, is active in both sexes of one species and also active in at least one sex of the second species and the second chemical that induces tumors at only one site and is active in one sex of one species) is a decile value of 3 (0.9 + 0.06 + 0.54 + 0.5 + 1.0) or a dose factor of 1000.

A major decision in deriving this activity scheme was the consideration of either averaging or selecting the individual adjusted sex-species decile values for calculating the carcinogen activity-F344 rat and the carcinogen activity-B6C3F$_1$ mouse or averaging or selecting the carcinogen activity-F344 rat and the carcinogen activity-B6C3F$_1$ mouse to calculate the carcinogen activity-combined. It was considered that for a carcinogen-activity scheme, positive decile values could be not be averaged with negative decile values as this might result in active chemicals being classified as inactive. Also considered was that an active chemical that had two dissimilar sex or species decile values (in numeric terms) should be represented in terms of its most sensitive indicator (i.e., the lowest TD_{50} value). Similarly, for inactive chemicals, the least sensitive HADD value was selected, which represented the higher of the two dose levels administered (the least toxic chemical). The impact on the selection process resulting from the rat being generally more sensitive than the mouse to the tumorigenic effects of chemicals is that the carcinogen activity-F344 rat was selected over the carcinogen activity-B6C3F$_1$ mouse in the calculation of the carcinogen activity-combined. Because the mouse is generally less sensitive than the rat to the toxic effects of chemicals, as evidenced by the HADD values, the carcinogen activity-B6C3F$_1$ mouse was generally selected over the carcinogen activity-F344 rat. The observation that the mouse is generally less sensitive than the rat to the carcinogenic and toxic effects of chemicals may be due to the protocol used by the NCI/NTP, which uses the maximum tolerated dose as the basis for dose selection.

In the future, this multifactor carcinogen-activity scheme could be modified in several ways. The values assigned to each of the factors could be altered to reflect a different emphasis. Additional factors could be incorporated into the scheme such as weightings for malignant tumors and weightings for the presence of specific metabolic, pharmacokinetic, and/or DNA adduct data that relate results in experimental animals to man. The selection of the F344 rat and the B6C3F$_1$ mouse as the test animals and the

oral routes as the standard routes of exposure places a restriction on the use of larger databases such as the "Survey of Chemicals which Have Been Tested for Carcinogenic Activity" (*14*) or the U.S. Environmental Protection Agency's Gene-Tox Carcinogen Data Base (*15*). These restrictions could be lifted under special circumstances to include strains of rats and mice other than F344 rats and $B6C3F_1$ mice and routes of administration other than oral.

The multifactor carcinogen-activity scheme presented here represents an initial effort in an investigation of methods needed to assess the potential risk of chemicals. It is hoped that this effort will catalyze other investigators into similar investigations towards modifying and improving the original concept presented. There is a pressing need for methods that can estimate the inherent carcinogenic activity of chemicals and a wide potential use for these methods with applications in both the research and risk assessment areas.

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency and no official endorsement should be inferred.

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