RApid Screening of Hazard Analysis[1]

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For synthetic fuels development, the Department of Energy was charged, in 1983, to "justify the need to monitor specific unregulated substances and of providing threshold values above which these substances must be monitored." In response, the RApid Screening of Hazard (RASH) method was developed as a non-expert, quick, chemical-scoring system to be used in the absence of compound-specific guidance from regulatory agencies.

By relative-potency comparisons, it is simple to compute the equivalent toxic dosage of the 'interviewing' compound (i.e., compound being evaluated) in terms of a standard reference compound. The process is analogous to the Relative Biological Effectiveness (RBE) factor used to compare different ionizing radiations. Mechanisms that underlie the responses achieved are not considered either in RASH or the analogy.

A basic requirement of RASH is that doses used in experiments on different compounds should cause equivalent biological response in a common bioassay. Standard rules for RASH evaluations were presented as simple default options that could be adjusted according to the user's knowledge. RASH was intended to use data such as those published in the Registry of Toxic Effects of Chemical Substances (RTECS). The objective was to measure an 'interviewing' compound against one or more reference compounds.

Most calculations based on RASH consider mutagenesis, carcinogenesis, reproductive toxicity, tumorigenesis, acute toxicity, and even irritation as reported in the RTECS, although the user could select. Thus, the typical result of the RASH process was to compute tentative exposure/monitoring concentrations (or doses) based on (a) chronic toxicity and/or (b) an assumption that the interviewing compound is carcinogenic either by its own properties or as a component of a complex exposure environment. One limitation of the RASH method is that it does not predict which compounds are initiators.

Carcinogenic testing depends on (1) whether the compound has all the properties of carcinogens and (2) specification of a test dosage adequate to potentiate frank cancer. Public concerns reduce all such considerations into the simple question of whether the 'interviewing' compound causes cancer. We have postulated that RASH provides a chemical scoring index of a compound's relative capacity to promote cancer through compensatory cellular proliferation. The present application was to evaluate whether doses used by the NTP were adequate (relative to a set defined median) to express cancer given Tennant's categories of "unlikely," "possible," and "probable" carcinogens.

From a decade of experience, it has been found that the compound-specific products of 'RP x Regulatory Benchmark' are reasonably constant for a variety of compounds evaluated by similar considerations (e.g., EPA-CAG, EPA-RQ, or ACGIH-TLV). Alternatively, the behavior can be viewed as an inverse proportionality between the median relative potency and the permissible exposure. After scoring more than 400 compounds, we observed (a) where both the interviewing compound and the reference compound(s) have been tested in about 10 to 20 common bioassays, it has been typical for RASH-derived concentrations to agree with estimates deriving from expert committees to within a factor of 2 or 3, (b) for about 5 to 10 relative potency values, factor of 10 agreement with expert evaluations have been common, and (c) for comparisons based on Ames tests with and without S9, factor of 100 accuracy has been the trend. Factor of 100 accuracy on a typical range of about 10^8 is useful for some considerations.

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Therefore, to predict the effectiveness of the 2-year test dosages (underlying Tennant's predictions) we made predictions only for tested compounds for which we thought the RTECS data were adequate to define a reasonably accurate estimate of whether the product of the median relative potency and test dose was adequate for carcinogenic potentiation--about 30 compounds for mice and 30 for rats. We posited that doses tested significantly below the bulk of the test-dose vs. relative-potency comparisons could have a low probability of revealing a positive carcinogenic effect even if those compounds would test positive at higher doses (false negatives). On the other hand, doses significantly above the preponderance of the test-dose vs. relative-potency comparisons might give false positive results through toxicity-associated compensatory cell proliferation. Additionally, we expected that the NTP result, [whether "no evidence" (NE), "equivocal evidence" (EE), "some evidence" (SE), or "clear evidence" (CE)] could vary with the magnitude of the test dose.

We supplemented the basic RASH method with:

- if "RP x Dose" is within a factor of two of the vertical medians in Figures 3 and 4 of Jones and Easterly (1991)--figures attached--we assigned "+" for Tennant's "probable" carcinogens, "-" for Tennant's "unlikely" carcinogens, and "Equivocal" or "E" for Tennant's "possible" carcinogens. Beyond a factor of two, the class was modified depending upon the direction of the deviation.

- if "RP x Dose" was 2-fold greater than the vertical line, the estimate based on Tennant's class was increased one level, i.e., "-" to "E", or "E" to "+", except for manganese sulfate monohydrate which was increased from "unlikely" to a "+" because of the large values of "RP x Dose" and "P_{25} x Dose".

- if the "RP x Dose" $<$ 1/2 value of vertical line, the prediction based on Tennant's class was decreased by one level as in "+" to "E", or "E" to "-", except where the "P_{75} x Dose" is not greater than the vertical line. There "probable" was changed to "-".

Overall, Tennant's predictions--without our modifications--for available results on the 44 compounds reflect $>$ 90% accuracy to predict a positive test result. Two disadvantages of the Tennant method seem to be: (1) the great amount of compound-specific and protocol-specific knowledge required to make predictions for new compounds, and (2) about 30% false positives.

As shown in attached figures, RASH modifications of Tennant's predictions yield only coin-flip accuracy. On the other hand, the accuracy to predict a true negative is nearly 90%.

At first impression, it seems that a method that can predict true carcinogens with a 9/10 accuracy could be coupled serially with other considerations that can predict true non-carcinogens with a similar accuracy so that a high degree of specificity could be achieved. Unfortunately, the false postives in one system and the false negatives in the other system prevent this accuracy.

References

Tennant RW, et al. **Mutagenesis** 5(1):3-14, 1990.

Jones TD and Easterly CE. **Mutagenesis** 6(6):507-514, 1991.

Reproduced from Figures 3 and 4 of Jones and Easterly (1991).

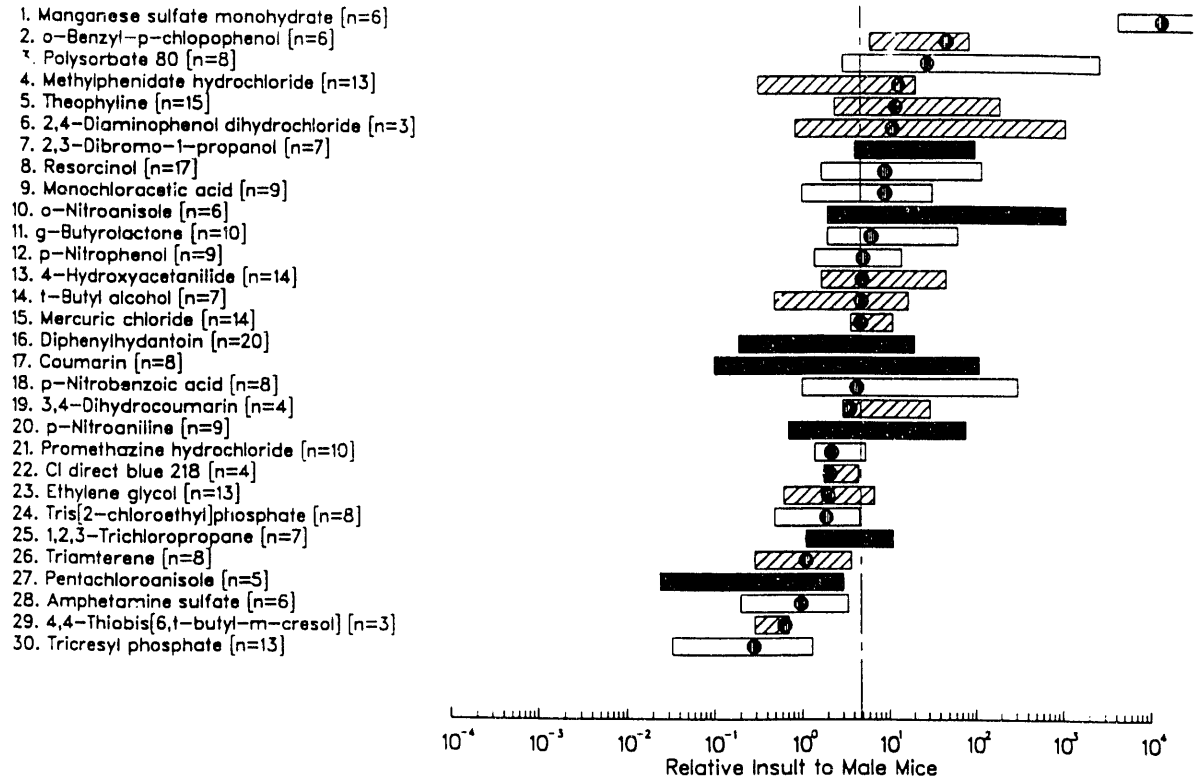


Fig. 3. Rank ordering of the predicted outcome from maximum treatment doses used by the NTP to test male mice. The solid bars are 'probable' carcinogens, the cross-hatched bars are 'possible' carcinogens and the open bars are 'unlikely' carcinogens (Tennant *et al.*, 1990).

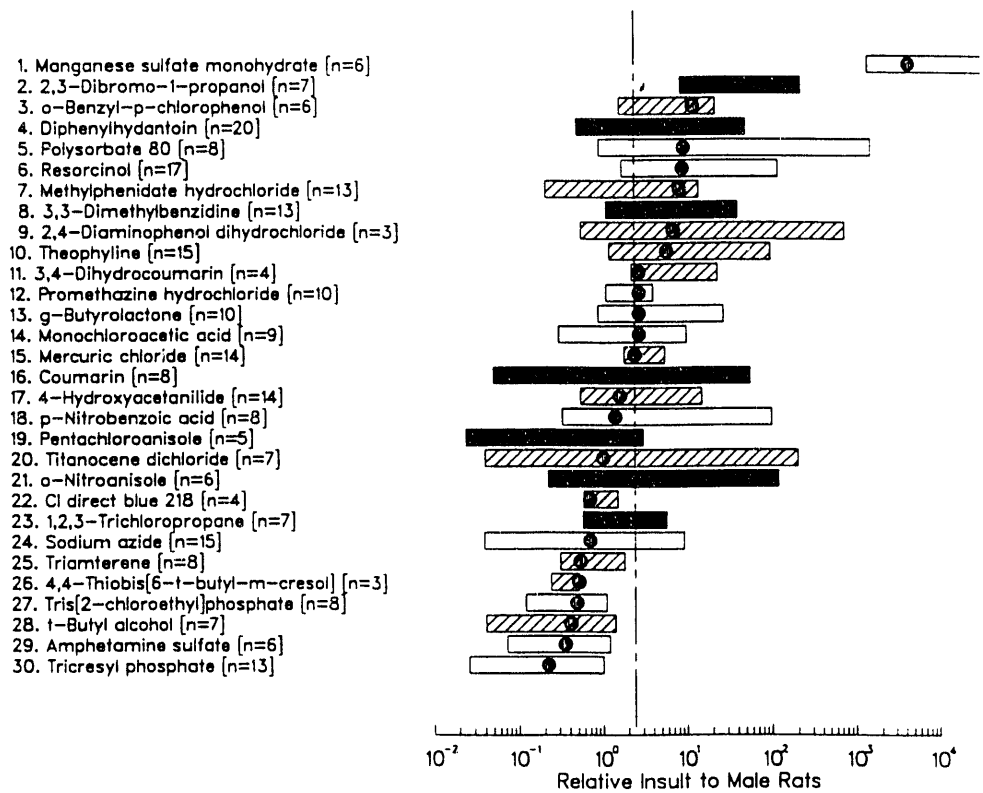
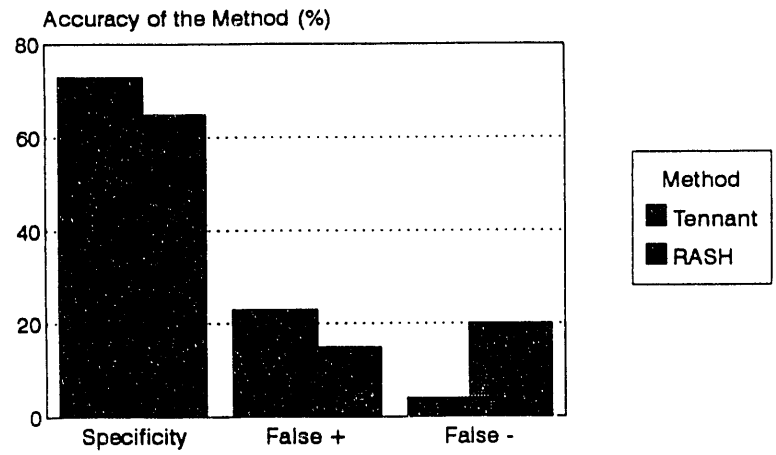


Fig. 4. Rank ordering of the predicted outcome from maximum treatment doses used by the NTP to test male rats. Bar patterns are the same as in Figure 3

Table 1. Comparison of NTP results, Tennant's Predictions, and Modifications by RASH for Male Rats and Mice.

		Male Rats			Male Mice		
	Compound:	**NTP**	**Tennant**	**RASH**	**NTP**	**Tennnant**	**RASH**
1.	Amphetamine sulfate	NE	-	-	NE	-	-
2.	Napthalene	NT	NT	NP	NE	-	NP
3.	Polysorbate 80	EE	-	E	NE	-	E
4.	Promethazine HCl	NE	-	-	NE	-	-
5.	Resorcinol	NE	-	E	NE	-	-
6.	γ-Butyrolactone	NE	-	-	EE	-	-
7.	Manganese sulfate monohydrate	NE	-	+	EE	-	+
8.	Monochloroacetic acid	NE	-	-	NE	-	-
9.	ρ-Nitorphenol	NT	NT	NT	NE	-	-
10.	Tricresyl phosphate	?	-	-	?	-	-
11.	o-Benzyl-ρchlorophenol	NE	+	+	SE	+	+
12.	2,2-Bis(bromomethyl)-1,3-propanediol	?	+	NP	?	+	NP
13.	τ-Butyl alcohol	?	+	-	?	+	E
14.	3,4-Dihydrocoumarin	SE	+	E	NE	+	E
15.	Ethylene glycol	NT	NT	NT	NE	+	-
16.	Mercuric chloride	SE	+	E	EE	+	E
17.	Methylphenidate HCl	?	+	+	?	+	+
18.	Theophylline	?	+	+	?	-	+
19.	4,4'-Thiobis(6-τ-butyl-m-cresol)	?	+	-	?	-	-
20.	Triamterene	EE	+	-	SE	-	-
21.	Diphenylhydantoin	EE	-	+	NE	+	+
22.	Pentachloroanisole	SE	+	-	SE	+	-
23.	Chloramine	NE	-	NP	NE	-	NP
24.	4,4'-Diamino-2,2'-stilbenedisulfonicacid,2Na	NE	-	NP	NE	-	NP
25.	Methyl bromide	NT	NT	NP	NE	-	NP
26.	ρ-Nitrobenzoid acid	?	-	-	?	-	-
27.	Sodium azide	NE	-	-	NT	NT	NT
28.	Tris(2-chloroethyl)phosphate	CE	-	-	EE	-	-
29.	CI Direct blue 218	SE	+	-	CE	+	-
30.	CI Pigment red 3	SE	+	NP	SE	+	NP
31.	CI Pigment red 23	EE	+	NP	NE	+	NP
32.	2,4-Diaminophenol2HCl	NE	+	+	SE	+	+
33.	4-Hydroxyacetanilide	NE	+	-	NE	+	E
34.	Salicylazosulfapyridine	?	+	NP	?	-	NP
35.	Titanocene dichloride	EE	+	-	NT	NT	NT
36.	CI Acid red 114	CE	+	NP	NT	NT	NP
37.	CI Direct blue 15	CE	+	NP	NT	NT	NP
38.	Coumarin	SE	+	+	SE	+	+
39.	2,3-Dibromo-1-propanol	CE	+	+	CE	+	+
40.	3,3'-Dimethylbenzidine2HCl	CE	+	+	NT	NT	NT
41.	HC Yellow 4	EE	+	NP	NE	+	NP
42.	ρ-Nitroaniline	NT	NT	NT	EE	+	E
43.	o-Nitroanisole	CE	+	E	CE	+	+
44.	1,2,3-Trichloropropane	CE	+	E	CE	+	E

Comparison of Tennant's Predictions with RASH Modifications of Tennant's "Unlikely," "Possible," and "Probable" Classes. Results are for Male Rats Combined with Male Mice.



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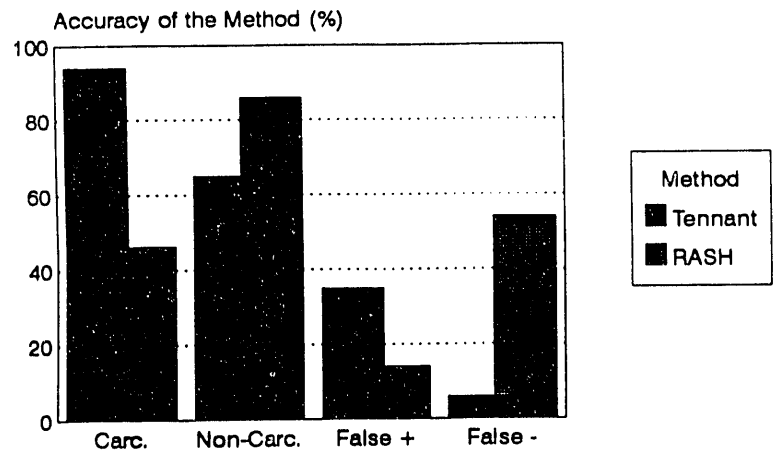

Table 2. Results from the RASH modification of Tennant's classifications listed by rodent test model.

Tennant's Classification	# Compounds Tested*	Accuracy of Method		False Positives		False Negatives	
		RASH	Tennant	RASH	Tennant	RASH	Tennant
No Positive Indications							
Male Rats	4/5	4/4	4/4	0/4	0/4	0/4	0/4
Male Mice	5/5	4/4	4/4	0/4	0/4	0/4	0/4
Non-Genotoxic, Unlikely Carcinogens							
Male Rats	4/5 (3)	2/3	3/3	1/3	0/3	0/3	0/3
Male Mice	5/5 (4)	3/4	4/4	1/4	0/4	0/4	0/4
Non-Genotoxic, Possible Carcinogens							
Male Rats	9/10 (4)	1/4	2/4	1/4	2/4	2/4	0/4
Male Mice	10/10 (5)	4/5	1/5	1/5	3/5	0/5	1/5
Non-Genotoxic, Probable Carcinogens							
Male Rats	2/2	0/2	2/2	1/2	0/2	1/2	0/2
Male Mice	2/2	0/2	1/2	1/2	1/2	1/2	0/2
Genotoxic, Unlikely Carcinogens							
Male Rats	5/6 (4)	1/2	1/2	0/2	0/2	1/2	1/2
Male Mice	5/6 (4)	1/1	1/1	0/1	0/1	0/1	0/1
Genotoxic, Possible Carcinogens							
Male Rats	7/7 (6)	2/4	1/4	1/4	3/4	1/4	0/4
Male Mice	6/7 (5)	2/3	2/3	0/3	1/3	1/3	0/3
Genotoxic, Probable Carcinogens							
Male Rats	8/9	3/5	5/5	0/5	0/5	2/5	0/5
Male Mice	6/9	4/5	4/5	0/5	1/5	1/5	0/5
TOTALS		32/48	34/48	6/48	11/48	10/48	3/48

* Value inside parentheses of column 2 is the number of compounds for which NTP results are available on 3/17/93.

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