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Evaluating consistency in the interpretation of NTP rodent cancer bioassays: An examination of mouse lung tumor effects in the 4-MEI study

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ABSTRACT

The potential carcinogenicity of 4-methylimidazole (4-MEI) was evaluated in a National Toxicology Program (NTP) rodent cancer bioassay in Fischer 344 rats and B6C3F1 mice (NTP, 2007; Chan et al., 2008). The NTP concluded that there was "clear evidence of carcinogenic activity" in male and female mice, based on an increased incidence of lung tumors. The "category of evidence" that the NTP assigns to a rodent cancer bioassay outcome can have significant regulatory implications. This is especially important for 4-MEI, which forms in caramel colorings and other foods during cooking, with potential widespread human exposure in a broad spectrum of food and beverage products. A detailed analysis of all NTP mouse-lung-tumor-only carcinogens reveals that the proper call for lung tumors in the 4-MEI study should have been "some evidence" rather than "clear evidence" of carcinogenic activity for both male and female mice in order to be consistent with the NTP's interpretation of other mouse lung carcinogens showing a similar strength of response. Suggestions are given as to measures the NTP should consider in the preparation of some or all future Technical Reports in order to enhance consistency of interpretation of experimental results.

1. Introduction

The National Toxicology Program (NTP) recently conducted a rodent cancer bioassay for 4-methylimidazole (4-MEI) in male and female Fischer 344 rats and B6C3F1 mice, and summarized the results in NTP Technical Report (TR) #535 (NTP, 2007) as well as in the scientific literature (Chan et al., 2008). The NTP concluded that 4-MEI showed "clear evidence of carcinogenic activity" in male and female mice, based on increased incidences of lung tumors. However, male rats showed no evidence of carcinogenic activity, while female rats showed only "equivocal evidence of carcinogenic activity". In fact, F344 rats receiving 4-MEI showed notable decreased incidences in a variety of tumors (Murray, 2010).

The NTP has two "categories of evidence" for studies regarded as showing carcinogenic effects: "clear evidence of carcinogenic activity" for the stronger effects and "some evidence of carcinogenic activity" for the weaker effects. The NTP defines these categories of evidence as follows: "clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy," while "some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign or combined) in which the strength of the response is less than that required for clear evidence". The NTP also has a classification for marginally increased tumor incidences that are considered to be uncertain findings: "equivocal evidence of carcinogenic activity". For an explanation of all NTP categories of evidence of carcinogenic activity, see http://ntp.niehs.nih.gov/?objectid=07027D0E-E5CB-050E-027371D9CC0AAACF.

The NTP definitions of "clear evidence" and "some evidence" allow for some flexibility in assigning a category of evidence classification to a given study. That is, it is a matter of scientific judgment whether or not an increased tumor incidence in a given study displays sufficient "strength of the response" to be considered "clear evidence of carcinogenic activity". Various entities, including some state and federal agencies, place considerable weight on NTP's assessments; thus, it is essential that the "strength of the response" criterion be applied uniformly over time in order to maintain a consistency in classification of NTP rodent carcinogens. Importantly, the categories of evidence are applied independently to each sex-species group (e.g., male mice; female mice); there is no category of evidence classification for the species as a whole.

The purpose of this article is two-fold: (i) to examine whether or not the "clear evidence" call made for lung tumors in the 4-MEI study is consistent with the calls made by the NTP for lung tumor effects in mice in other similar studies; and (ii) to present

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suggestions for the NTP to consider in the preparation of future Technical Reports to enhance consistency in the interpretation of experimental results.

2. NTP database evaluated

The first step in the evaluation was to examine all NTP studies showing lung tumor carcinogenicity in mice. This information is available at http://ntp-server.niehs.nih.gov/index.cfm?objectid=0723BF27-A528-5BA3-093569186D34B7BA. The second step was to discard the studies that were evaluated before the NTP's current "categories of evidence" paradigm. The third step was to focus on those studies in which the only evidence of carcinogenicity in male or female mice was lung tumors. This was done to maintain an "apples to apples" comparison, because if carcinogenicity were seen at multiple sites, it would be unclear the extent to which the lung tumors contributed to the overall call.

The relevant lung tumor rates were then summarized for each study, with separate listings for lung adenoma, carcinoma, and adenoma/carcinoma combined. The NTP gives primary emphasis to lung adenoma/carcinoma combined, since the dividing line between the diagnosis of adenoma and carcinoma may be somewhat subjective (as discussed below). Nevertheless, it was of interest to examine benign and malignant lung tumors separately to determine if this had an impact on the overall interpretation of the data.

The goal of this evaluation was to answer the following questions: (i) for NTP carcinogenicity studies in male or female B6C3F1 mice that showed carcinogenic effects only in the lung, and using a weight of evidence approach that considered p values, dose–response, survival, historical control data, pre-neoplastic lesions, etc., was the pattern of tumor increases seen in the 4-MEI study more like the "some evidence" lung carcinogens or the "clear evidence" lung carcinogens? and (ii) might the NTP benefit by including in certain of its Technical Reports information on how similar patterns of tumor occurrence have been interpreted in previous studies?

3. Results

3.1. "Clear evidence" carcinogens

This evaluation found six "clear evidence" mouse lung carcinogens, and these are summarized in Table 1 (males) and Table 2 (females) (NTP, 1990, 1998, 2002b, 2003, 2009, 2011). Five were inhalation studies, and one (riddelliine) was a gavage study. These tables include the genotoxicity data as reported by the NTP (+, positive; −, negative; M, mixed positive and negative), as well as the historical control lung tumor rates as given in each TR.

Tetranitromethane, vanadium pentoxide, and cumene clearly showed much stronger lung tumor effects (in both males and females) than did 4-MEI (see Tables 1 and 2).

Riddelliine (which was tested at only a single dose in female mice) showed a significant lung tumor effect in females, accompanied by markedly ($p < 0.001$) reduced survival in the single dosed group used in this study relative to controls. The majority of lung tumors observed in dosed riddelliine female mice occurred in animals dying before the end of the study, whereas all but one of the lung tumors observed in the high dose 4-MEI female mice occurred in the survivors. The NTP did not identify the cause of the early deaths in the riddelliine female mice; it is possible that lung tumors contributed to the early death seen in this group, although increased mortality was also observed in the high dose male mice without an elevation in lung tumor occurrence.

Importantly, because of the early deaths, female mice dosed with riddelliine had less opportunity for tumors to develop than did the high dose female mice in the 4-MEI study. That is, although the overall lung tumor rates were similar in the dosed riddelliine (13/50) and high dose 4-MEI (14/50) female mice, the survival rates were significantly ($p < 0.001$) different: there were only 17/50 survivors in the dosed riddelliine female mice compared with 40/50 in the high dose 4-MEI female mice. Had more dosed female mice survived in the riddelliine study, the lung tumor rate would almost certainly have exceeded (perhaps substantially) the lung tumor rate observed in the high dose 4-MEI females.

A survival-adjusted statistical analysis revealed that the lung adenoma/carcinoma effect observed in the female mice receiving riddelliine ($p < 0.001$; actually even stronger, but this is the limit of p values reported by the NTP) was more significant than the high dose lung adenoma/carcinoma effect seen in the 4-MEI study ($p = 0.002$).

1-bromopropane, like riddelliine, showed increased lung tumor incidence only in females, but unlike riddelliine, there were no survival effects. The carcinogenic effects in female mice receiving 1-bromopropane were stronger than in the 4-MEI study. For example, the statistical significance of the trend (and the high dose effect) for adenoma/carcinoma combined for 1-bromopropane was $p < 0.001$ (probably even stronger, but this is the limit of statistical significance reported by the NTP) compared with $p = 0.002$ for 4-MEI. Moreover, there was a stronger effect on carcinoma (alone) in the 1-bromopropane study.

In all three 1-bromopropane female mice dosed groups, the incidences of lung carcinoma (7/50, 5/50, 4/50; see Table 2) fell outside the historical control range for that study (0–6%) compared with a zero incidence in the concurrent control group. For the 4-MEI study, only the top dose incidence (7/50) fell outside that range, and it was not even statistically elevated relative to the concurrent control lung carcinoma rate (3/50). Overall, the incidence of lung carcinoma was 11% (16/150) in the (pooled) 1-bromopropane females compared with 0% (0/50) in the controls. In contrast, the incidence of lung carcinoma in the pooled 4-MEI females (9/150, 6%) was exactly the same as the rate seen in the concurrent control group (3/50, 6%).

The lung tumor effect in the cobalt sulfate heptahydrate study (which showed no survival effects) was also stronger than that in the 4-MEI study. The high dose effect for lung adenoma/carcinoma was stronger statistically in the cobalt sulfate study for both males ($p < 0.001$ vs. $p = 0.003$) and females ($p < 0.001$ vs. $p = 0.002$) than in the 4-MEI study. Moreover, for female mice the lung carcinoma high dose effect was more statistically significant in the cobalt sulfate heptahydrate study than in the 4-MEI study ($p = 0.009$ vs. $p = 0.154$); the corresponding p values were similar for males: $p = 0.033$ vs. 0.042. There were also much stronger non-neoplastic lung lesion effects in the cobalt sulfate heptahydrate study than in the 4-MEI study, which lends additional support to a weight of evidence approach to lung carcinogenicity. Thus, the overall evidence for lung tumor carcinogenicity in mice was stronger in the cobalt sulfate heptahydrate study than in the 4-MEI study.

Thus, the lung tumor effects observed in male mice in the 4-MEI study are weaker than those seen for male mice in any of the four "clear evidence" NTP "lung only" carcinogens summarized in Table 1. Similarly, the lung tumor effects observed in female mice in the 4-MEI study are weaker than those seen for female mice in any of the six "clear evidence" NTP "lung only" carcinogens summarized in Table 2. This does not necessarily imply that 4-MEI was "over-classified", because we have not yet compared the pattern of lung tumor response seen in the NTP "some evidence" mouse lung carcinogens with those seen in the 4-MEI study. These comparisons are given below.

3.2. "Some evidence" carcinogens

The evaluation also found six "some evidence" mouse lung carcinogens, and results for these studies are summarized in Table 3