Reviewer's report

Title: N-nitrosomethylurea in the mammary gland: is there a non-carcinogenic dose?

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Reviewer: Tommaso Dragani

Reviewer's report:

The manuscript of Murray et al., describes the results of a rat mammary carcinogenicity study with N-nitrosomethylurea (NMU). The authors have carried out a dose-response study using 4 doses of carcinogen: 10, 20, 30, and 50 mg/kg body weight (bw). The authors have carried out detailed analysis of gross and microscopic neoplastic lesions and concluded that “A true “non-carcinogenic” NMU dose may be difficult to define”. However, this statement is not based on their results. Indeed, (i) they have analyzed only a few doses of NMU and, most likely, the inclusion of doses below 5 mg/kg bw would have provided evidence of threshold; (ii) they have not carried out an adequate statistical analysis of the results.

As it has been pointed out in numerous publications, linear (arithmetic) scale for the dose of chemicals obscures effects at doses below those used in the experiment and distorts the effect seen over the range of doses used. It has been proposed that, because of thermodynamic reasons, a logarithmic relationship of dose to biological effects must be used [Waddell WJ. Thermodynamic basis for expressing dose logarithmically. Toxicol Appl Pharmacol. 2008 Apr 15;228(2):156-7]. By such type of analysis, a threshold has been observed for many chemical carcinogens [Waddell WJ. Thresholds in chemical carcinogenesis: what are animal experiments telling us? Toxicol Pathol. 2003 May-Jun;31(3):260-2], and a threshold could also be observed by plotting the data of Table 3 of Murray et al., i.e., logarithm of the dose versus incidence of microscopic lesions. Such a threshold is estimated to be at just below 10 mg/kg bw NMU, and if the authors had carried out an experiment at 3 mg/kg bw NMU, most likely they would have observed a no-effect level of NMU on rat mammary carcinogenesis.

Therefore, the authors must quote the relevant papers of Waddell WJ and rewrite accordingly the Abstract, Discussion and Conclusion sections.

Overall, this is an interesting manuscript that could be greatly improved by some relatively simple changes.

Minor points:

1. Latency must be analyzed using time-based statistics that are used to test survival (e.g., Kaplan-Meier curves and log-rank test, Cox’s analysis). Analysis of tumor latency by ANOVA is a mistake.

2. Tables 1 and 2 must be deleted and the relevant information should be moved
to the Results.

3. Results of Table 3 at 25-30 weeks could be plotted in a nice dose-response graph, as I have drawn by reporting the dose in logarithm units (0 dose excluded) versus incidence of microscopic lesions, and adding a linear fitting of the data (r=0.94).

4. Tables 4 and 5 must be either deleted and their data briefly reported in the text, or summarized in a single Table reporting the dose-response overall data.

5. Figures 1B and 1C must be deleted since mammary tumors of thoracic or abdominal origin are the same biological entity.

6. Figure 2 must become Figure 1B.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.