Reviewer's report

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

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Reviewer: Denise Twinn

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This study set out to define a dose response relationship for inducing mammary tumours in the Wistar-Furth inbred rat strain with the widely used carcinogen n-nitrosomethylurea (NMU). Virgin female rats of 49-58 d of age were injected with doses of 10, 20, 30 and 50 mg/kg bodyweight NMU and the resulting palpable, and microscopic lesions were followed at 12, 22 and 25-30 weeks post-injection. An increase in tumour burden and decrease in latency was observed with increasing doses, but only for the higher doses of 30 and 50 mg/kg. 30 and 50 mg/kg doses of NMU resulted in both neoplastic and pre-neoplastic structures as early as 12 weeks post induction. Interestingly, preneoplastic aberrant structures were described and observed for the 2 lower doses (10 and 20 mg/kg bw) and these included a pregnant/lactational gland phenotype with milk production, sloughing of epithelial cells into the lumen, increased collagen expression in the stoma surrounding ducts, hyperplastic structures and DCIS structures. These by and large did not progress to palpable tumours within the time course studied. The authors suggest, as have others in the past, that tumour incidence and latency is strain dependent and propose that the lower doses may be used as a threshold model (described as “baseline” by the authors) for studying the impact of environmental or other additional insults for altering susceptibility.

The paper is well written and the question well defined. Methods are appropriate and well described. The data is sound and conclusions are balanced and supported by the data. The abstract is clear and concise and the title is appropriate. Similar studies have been carried out in the past, but this paper takes a further step in characterising not only the effects of dose on latency and incidence but sets out to clearly define the properties of pre-neoplastic microscopic lesions.

Minor Revisions are recommended as follows-

1. Could authors comment on the apparent delay in incidence of inguinal palpable tumours occurring in 100% of animals given the 50mg dose compared to the thoracic tumours? (21w for inguinal vs. 16w for thoracic).

2. It is worth mentioning the mechanism of action of NMU, i.e. its effects on DNA, why it targets mammary tissue and salivary tissue, but not other tissue types.

3. Could the authors comment on why the pre-neoplastic structures observed for
the lower doses apparently do not progress to neoplasia? Would it therefore be more appropriate to call these lesions dysplastic rather than pre-neoplastic?

4. Mammary glands from one side were excised at 12w and the contralateral side at 22w. While this allows pairing of the data, the total incidence at each time point is being underestimated approximately by a factor of 2 (if we assume an approximately equal number of lesions to form on either side) compared to the final time point of 30w.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests