CORRESPONDENCE

Studies of the carcinogenesis and tumourigenesis of skin applications of dodecylbenzene on hairless mice

Sir,—Several aspects of the study reported by Iversen (1989;46:608-16) give rise to concern, including its design and conduct, the absence of dose response relations, and the marginal statistical significance observed.

Iversen alludes to problems of potential misdosing of animals but dismisses the potential significance for the overall results. Individual identification of animals was performed only after the development of a lesion, not from the start of the study. Animals were not individually housed. The incidence of papillomas in the negative control animals is unusual, although this had not been observed previously in Iversen’s laboratory. We contend that any misdosing or misassignment of animals could have been significant to the overall outcome of the study, particularly since the dose of DMBA used (51.2 mg) for the positive control mice caused about half of the treated animals to develop skin tumours. We consider that the incidence of tumour bearing animals in this case may have been too high to distinguish clearly promoting activity.

The incidence of malignant lymphomas in negative control animals was 3.6% (2/56), in the low dose group 3.6% (2/56), and in the high dose group 10.7% (6/56). Comparison of the controls and the high dose group was not considered statistically significant (0.10 > p > 0.05 by a chi-squared assessment using one tailed test). Therefore no clear evidence is presented that malignant lymphomas were related to treatment. As Iversen notes, hairless mice are known to develop reticuloses in old age. High incidences of lymphoid leukemias have also been reported in these animals. The Norwegian Cancer Registry found no excess in lymphomas or leukemias in a large cohort of workers exposed to mineral oils and to alkylbenzenes (O H Iversen, personal communication).

Dodecylbenzene (a branched chain alkylbenzene) is a skin irritant in mice. Iversen describes lesions including ulcerations and pronounced hyperplasia. The interpretation of results from dermal bioassays conducted under such conditions is important. Repeated epidermal regeneration can elicit promotion of both benign and malignant neoplasms in mouse skin. Disruption of the barrier function of the skin may potentially invalidate a chronic cutaneous study.

We have conducted an eight week dose range-finding study in C3H mice using two materials (C10-C13 linear alkylbenzenes) of similar irritant potential to that of dodecylbenzene (branched chain alkylbenzene) (ECOSOL unpublished data). The study was conducted to GLP and used various treatment regimens. Gross and histopathological examinations of the treated skin were assessed according to the US-EPA criteria for setting the maximum tolerated dose. This was assessed as 5% linear alkylbenzene (in acetone) applied twice weekly. This conclusion was corroborated by an ad hoc group of pathologists, two of whom were members of the EPA workshop on dermal toxicity. We therefore conclude that at each of the concentrations used by Iversen (16%, 40%, and 80%) the maximum tolerated dose was exceeded.

Iversen’s conclusions from mouse bioassay data are not supported by results of in vitro studies conducted recently (and A L Meyer, unpublished data). Alkylbenzenes (branched chain and linear) with major components in the C9 to C12 range show overwhelming negative genotoxic and cell transforming activities.

In evaluating the significance to man of Iversen’s findings, we agree that no evidence is presented to indicate that dodecylbenzene (branched chain alkylbenzene) poses a carcinogenic hazard to man. We believe that the tumourigenic response to 80% dodecylbenzene is related to compromised skin. Lower levels of dodecylbenzene provide no evidence of such an effect. We agree that there is no evidence of a carcinogenic response and, finally, we do not believe that the observations of malignant lymphomas were related to treatment.

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Author’s reply:

The authors of the letter think that my study “gives rise to concern” as regards the design and conduct, the absence of a dose response relation, and the marginal statistical significance observed. The design and conduct of my study were carefully worked out. The alkylbenzene tested was not a linear, but a branched compound. A research and testing services contract was signed in September 1983 by myself and vice president R Wilson of Essochem Europe. During the study we were inspected by two auditors, one from the quality assurance division of Exxon Corporation (J K Baldwin, USA) and one from Essochem Europe (N J Sarginson). They went through the whole experiment, the laboratory books, the identification of the animals, etc, and found everything acceptable.

I presume that the authors of this letter are not seriously intending to suggest that we deliberately misdosed the animals to obtain a desired result or to cast doubt on the quality or scientific integrity of the laboratory and its researchers and technicians. The study was personally prepared and applied; the initial dose of DMBA and the technicians applied the oils and fluids twice a week by an automatic pipette. On page 611 of my paper it is specifically mentioned that there were no statistically significant differences in tumour rates or yields between the group of 48 animals that received DMBA once in this study and all the other groups at our institute that had received one application of the same dose of DMBA. This is what I meant by saying that “any possible small misdosing of animals is of no significance for the overall results.”