

Pathology Review of Carcinogenicity P020023

The device contains 1,4-butanediol diglycidyl ether, a compound that has been shown to be mutagenic in various assay systems and was associated with tumor formation in mice following topical application in one animal study.

Four groups of CFI Mice (Shell Toxicology Laboratory) (6 months old at commencement of treatment, 50 per sex per group, 100 per sex in the vehicle control) were administered twice weekly topical applications of the test articles and control (0.2 ml. per dose) for 103 weeks. The first group received the vehicle, acetone. A second group received beta-propiolactone (2% in acetone). The third and fourth groups received 0.05% and 0.2% BDDE in acetone.

Animals were observed daily for clinical signs and appearance of the skin. The date of appearance, size and position of each cutaneous nodule was recorded.

After 10 weeks, surviving animals were sacrificed by phenobarbitone. Gross and microscopic examinations were conducted on all animals on the study. Histopathological examinations were performed on the following tissues: cutaneous nodules, liver, kidneys, spleen, pancreas, stomach, gonads, uterus, bladder, heart, lungs, thymus, adrenals, pituitary, thyroid, salivary glands, submaxillary lymph node and brain. Diagnosis of all skin tumors was confirmed by histopathology.

Results

- ?? Survival of control male and female animals was 26% and 22%, respectively. Survival of males was 26% and 34%, low and high dose, respectively, and females, 34% and 18%, respectively. Survival of treated groups was comparable to controls and in basic agreement with most international guidelines.
- ?? The most frequently recorded clinical signs were cachexia, cutaneous/subcutaneous masses and abdominal distension. However, none were observed in an incidence greater than that of controls.
- ?? The incidence of causes or contributors to premature death in mice treated with either 0.05% or 0.2% BDDE did not differ significantly from the vehicle control group.
- ?? Vehicle induced cutaneous lesions amounted to 16/99 males and 14/99 females which included scabbing and ulceration. Induced cutaneous lesions in males was 7/50 and 16/50 (0.05% and 0.2% doses, respectively) and in females was 5/49 and 8/50 (0.05% and 0.2% doses, respectively). A higher incidence of irritant lesions, characterized by

epilation and skin flaking/scabbing compared with the controls was induced only in the high dose males.

?? Exposure to BDDE did not result in an increase in the number of epidermal cell layers compared with the acetone controls. Two tumors were observed at the treated site, one in high dose male (subcutaneous fibrosarcoma) and high dose female (basal cell carcinoma). Four types of tumors distal to the treated site were observed in dosed animals; two basal cell carcinoma (one on the buccal cavity and another in the ventral neck) in a low and high dose female and an adenocarcinoma of Zymbaf's gland in a high dose female and a fibrosarcoma of the subcutis the axilla in a high dose male and female. Because of the low incidence of these lesions and a lack of dose relationship, it is concluded that these observations are unrelated to treatment. These conclusions are in agreement with the original study conclusions and the conclusions of the senior pathologist, Dr. Gopinath, who also reviewed this study. The exception was the observed increased incidence of lymphoblastic lymphosarcoma (non-thymic origin) in treated female mice. However, Dr. Gopinath, in his review, points out that, according today's standards, lymphoblastic lymphosarcoma is not a term that is currently used. Those tumors are now considered Lymphoid tumors and are grouped with what was previously termed, reticulum cell sarcomas and lymphocytic lymphosarcoma. When that is accomplished with respect to the data in this study, there is no longer an observed increase in dose related tumors with respect to controls. As Dr. Gopinath points out in his review, the incidence of lymphoid tumors in historical controls of female mice is very high.

?? The sponsor concluded that Exposure of CF-1 mice to topical doses (4 or 16 mg/kg) of BDDE twice weekly for 103 weeks did not result in any treatment related effects.

The systemic tumor data, however, indicated some disparity in the incidence of lymphoblastic lymphosarcoma limited to females only, but there was no conclusive evidence to infer that DBBE is a systemic carcinogen for CFI mice when applied topically to dorsal skin. In the study the female mice with lymphoblastic lymphosarcoma showed a statistically significant increased incidence and a positive trend. This effect was seen with both thymic and nonthymic lymphoblastic lymphosarcomas (separately or together). The effect was seen only among female mice.

Table1 Incidence of lymphoreticular tumors in female mice

	Acetone	B-PI	0.05% DBBE	0.2% DBBE
Reticulum cell sarcoma	16	4	12	4

Lymphoblastic lymphosarcoma	2	3	3	5
Thymic lymphoblastic				
Lymphosarcoma	3	2	1	5
Lymphocytic lymphosarcoma	1	1	1	0
Stem cell leukemia	2	0	0	2
Mice examined 100	100	50	49	50

The incidence of lymphoblastic lymphosarcoma showed an increase compared to the control in females. The classification of lymphoma used in the study is not currently used in toxicological pathology. The analysis used in the study is incorrect due the separation of lymphoid tumors in the analysis. Current thinking regarding lymphoid tumors(malignant lymphoma) is to summate all subtypes of lymphocyte derived **neoplasms** together for all analyses. Taking this into consideration, the lymphoid tumors in this study should include all subtypes including reticular cell sarcoma and lymphocytic lymphosarcoma along with lymphoblastic lymphosarcoma (both thymic and nonthymic). This necessitates a retabulation as follows:

Table 2. The incidence of lymphoid tumors(malignant lymphoma) in female mice.

	Acetone	B-PI	0.05% BDDE	0.2% BDDE
Total Lymphoid Tumor (malignant lymphoma)	22	10	17	14
Mice examined	100	50	49	50

As seen in table 2, the incidence of malignant lymphoma showed no significant variation from the control **group** and furthermore, showed no dose relationship.

Malignant lymphoma in mice is a common spontaneous tumor type with a highly variable incidence (Maita et al 1985). Viruses are known to induce murine lymphomas. Factors such as stress, housing (single vs gang), diet and time; related genetic drifts are known to affect the incidence of lymphomas in mice (Peristianis et al 1988, **Gopinath** 1994). In addition there are reports of other studies in mice with DBBE - based resins which revealed no effect on lymphoid tumors(Peristianis et al 19:38, Zakova et al 1985, Holland et al 1981). Considering all the factors mentioned and on weight of evidence; the study has not shown any treatment related effect on the incidence of lymphomas in mice of either sex.

Conclusion

I agree with sponsor's explanation. All the lymphomas should be taken together. To sum up the lymphoid tumors of each group into Table 2 seems reasonable.

I analyzed the association in cancer rate between control, 0.05 % BDDE and 0.2 % BDDE by using Chi-square test (SAS. Version 8). There is no statistically significant difference in cancer rate among treated groups and control. The incidence of lymphoma of each group was not dose related.

	Acetone	B-P1	0.05 % BDDE	0.2 % BDDE
Acetone	p=0	P=0.78	P=0.10	P=0.42
B-P1	P=0.78	P=0	P=0.1	P=0.34
0.05 % BDDE			0	P=0.47
0.2 % BDDE				0

There is also caveat that the BDDE is mutagenic in Ames test and other genotoxicity assays. I cannot conclude that lymphomas in this study were caused by BDDE.