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FROM: T. G. Grumbles
DATE: ~~January 1, 1980~~ 9-30-87

Interoffice Communication

SUBJ: MONSANTO FYI TSCA SUBMITTAL: DODECYLBENZENE TOXICOLOGY STUDY

VISTA

Attached is a copy of the paper that Monsanto submitted to EPA. Jim Mieure says their toxicologist has had no luck in trying to get more information regarding this report.

Thomas G. Grumbles

T. G. Grumbles

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Attachment

cc O. C. Kerfoot
A. M. Nielsen
C. M. Starks

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FINAL REPORT ON THE CHARACTERIZATION OF THE CARCINOGENIC NATURE OF DODECYLBENZENE AS DELIVERED BY ESSOCHEM, IDENTIFICATION CODE NO. MRDE-7

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The conditions of the test were formulated in a Research and Testing Services Contract between Essochem Europe and Universitetets Institutt for patologi, Rikshospitalet, Oslo, signed by R Wilson on behalf of Essochem on August 26th 1983, and by Olav Hilmar Iversen on behalf of the Pathology Institute September 13th 1983. Several interim reports have been submitted during the study.

MATERIALS AND METHODS

The idea was to test the carcinogenic potency of Dodecylbenzene using skin applications on hairless mice. Dodecylbenzene in vehicle should be given in an amount of 50/ul twice a week. Some groups of mice consisted of 56 animals, 28 males/females, others consisted of 48 animals, 24 males/females (see below).

Test animals used were hairless mice, hr/hr Oslo strain, purchased from Gamle Bomholt Gaard, Aarhus, Denmark.

DMBA was from Eastman Organic Chemicals, Rochester, N.Y., USA All acetone used was of reagent grade. The Dodecylbenzene was delivered by Essochem and was dissolved in reagent grade acetone.

Both DMBA and Dodecylbenzene were given in various concentrations with a graded pipetter.

One negative control group (1.) (56 mice) was painted with 50/ul reagent grade acetone twice a week. One positive control group (4.) (48 mice) was given one single application of 51.2/ug DMBA in acetone. A third group (2.) (56 mice) was painted with 16% and another one (3.) (56 mice) with 80% Dodecylbenzene twice a week, and finally a group (6.) (48 mice) was first given 51.2/ug DMBA and thereafter continual treatment twice a week with 40% Dodecylbenzene.

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CRITERIA FOR HISTOLOGICAL ASSESSMENT

By histological investigation it is easy to decide when there is an ulceration, a pronounced hyperplasia, excess pigmentation, dermatitis, or whether it is a benign papilloma or a squamous cell carcinoma or other form of malignancy in the skin. Sometimes it may be difficult to distinguish between a spindle cell sarcoma and a spindle cell squamous carcinoma. In those cases where one could see clear connection between the epithelium and the spindle cell tumor, these tumors were classified as spindle cell squamous cell carcinomas. In the cases where there was connective tissue between the tumor and the epithelium, the tumor was characterized as a sarcoma of the skin.

The spleen was often enlarged, often hyperplastic, and sometimes changed by amyloidosis, which is a degenerative lesion with precipitation of a protein substance. Amyloidosis was sometimes also found in the kidneys and in the liver.

Lung adenomas are known to be produced by many carcinogenic substances, especially urethan. In a control study at this laboratory we have found a spontaneous frequency of about 2-3 lung adenomas per 56 mice.

In swollen lymph nodes and swollen spleen we found some malignant lymphomas. As known from rodent pathology, collection of plasma cells in the spleen or lymph nodes should be registered as reactive changes and not as plasmocytomas. Sometimes lymphocytic infiltration was seen in the lung, in the liver and in the kidneys, indicating spread of lymphomas.

When there was an obvious proliferative lesion in lymph nodes, but not an obvious malignant lymphoma, the lesion was classified as a reticulosis. The criteria for diagnosing malignant lymphomas/leukemia in mice have been described by S. Wogan: "Tumors of the mouse hematopoietic system. Their diagnosis and interpretation in safety evaluation tests. Report of a study group. CRC Crit. Rev. Toxicol. 13 161-181, 1984".

OBSERVATION

The animals were painted twice a week and observed in connection with the painting. In the DMBA alone group the animals were observed once a week. The mice were observed weekly for skin lesions or swollen lymph nodes. All mice that died or were killed were autopsied. Skin and inner organs were inspected. If any pathological lesion was found, lungs, liver, spleen, swollen lymph nodes and kidneys were embedded in paraffin. Histological sections were made and the slides were studied microscopically.

DOCUMENTATION

The following documentation was used:

A list with a description of the experiment and the number of the experiment, and what was applied to the animals. Each mouse on these lists was given a number and allocated to a cage with 8 mice in each. Altogether we had 33 cages with 8 animals in each.

Each cage was labelled with number of mice, sex, birth date, treatment type and cage number.

Within each cage each single mouse got an individual number, and in the laboratory books each mouse was given at least one page. On each page in the book two outlines of a mouse were stamped, one for the backside and one for the abdominal side. Tumors were charted and noted by text.

As soon as a mouse got a lesion, it was given a particular number. Mice no. 1 in a cage was cut in the left ear, no. 2 was cut in the right ear, no. 3 in both ears, and no. 4 remained untouched. No. 5 was cut in the tail and in the left ear, no. 6 in the tail and in the right ear, no. 7 in the tail and both ears, and finally mouse no. 8 in each cage had only its tail cut. In this way the mice were labelled individually and could be identified at each observation. We have found this way of identifying mice better than fixing a metal number to their ears, since such numbers can easily fall off.

In addition to the first mentioned list and the laboratory books, there was also for each experiment produced a big table on graph paper in which each single mouse had a horizontal line and each week was designated on top of a vertical column. When skin lesions or swollen lymph nodes occurred this was drawn on the table, and from the start of the line for each tumor and onwards in time a line was drawn. When a mouse died this was marked with a cross, and at the end of the line the most important histology diagnoses were written.

The whole experiment was performed under the responsibility of Professor Olav Hilmar Iversen, and the Head of the Laboratory for Experimental Animals, Dr.med.vet. Leif Schjerven. A great amount of the practical work was made by a student (Marit Berg), and from October 1984 by a veterinary student (Inge Engeland). The personell in the Animal Laboratory also took care of the mice, changing in the cages, providing fresh water and food.

STATISTICAL EVALUATION

On the basis of the crude incidence of skin tumors, statistical investigation was made. The results are presented as tumor rates (the percentage of tumor-bearing animals in relation to the number of animals alive at the appearance of the first tumor related to time) and tumor yields (the cumulative occurrence of all skin tumors related to time) in all groups.

To evaluate differences in tumor rate, we have used the method for "non-incidental" tumors described by Peto and elaborated with a computer-based test program by Peto et al. This program takes into account varying mortality rates among the experimental groups, and assesses both the number of tumor-bearing animals and the time of the first tumor in each animal.

To evaluate the differences in tumor yield, we have used the method of Gail et al. based on "multiple times to tumor", Method 3. This method assesses the number of tumors appearing, varying mortality between the groups, and the time of appearance of each tumor.

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Finally the numbers of malignant skin tumors, lymphomas and lung adenomas in each group were analyzed with the Chi-square test. Since these variables may not be normally distributed, the Chi-square analysis is only a rough estimate.

RESULTS

Skin tumors

These are shown in Table 1 and in Figs. 1 and 2. Statistical evaluation is shown in Table 2. By tumor rate is meant the percentage of tumor-bearing animals in relation to the number of animals alive at appearance of the first tumor in relation to time, and by tumor yield is meant the total number of tumors calculated for 56 starting mice. Hence, the values found for the groups with 48 mice have been multiplied with 56/48 = 1.1667. These represent the values that would have appeared in a group of 56 mice with the same tumor yield as that observed in 48 mice. When the experiments are ranked as shown in Table 2, first column, there is a very significant trend. As to the positive control groups shown (Groups 4 and 5), we have for statistical investigation used both the control group of this study, plus a control group comprising all mice which in this institute have been painted once with 51.2 ug DMBA, altogether 128 mice. It is obvious, as can be seen from Table 1 and the curves that there can be no statistically significant difference between the effects of acetone alone and those of 16% Dodecylbenzene. They have therefore not been statistically tested. There is, however (Table 2), a significant difference between acetone alone and 80% Dodecylbenzene, but no difference between 16% and 80% Dodecylbenzene. There is a very significant difference beteween acetone and DMBA initiation, but there is only a suggestive difference between DMBA alone and DMBA followed by 40% Dodecylbenzene as regards tumor-bearing animals, and tumor yield when concurrent controls are used. There is a significant difference in total tumor yield for DMBA plus 40% Dodecylbenzene when compared with historical controls

Hence, as regards skin tumor it can be concluded that 80% Dodecylbenzene alone is a weak, complete tumorigen, whereas if 16% Dodecylbenzene has a carcinogenic potency, it is below measurable level. As regards 40% Dodecylbenzene as an enhancer, there is a weak promoting activity.

Lymphoid tumors

Eighty per cent Dodecylbenzene gave 6 lymphomas in 6 animals, whereas the acetone group gave only 2. DMBA alone and DMBA followed by Dodecylbenzene gave 5 and 4, respectively. If reticuloses are also counted, there seems to be no significant increase in lymphoma incidence.

Lung adenomas

For lung adenomas there is no significant difference between controls and Dodecylbenzene-painted animals, and the same refers to other tumors occurring.

Amyloidosis

As to the degenerative lesion amyloidosis, there is a significant increase in amyloidosis after 80% Dodecylbenzene. The explanation for this is difficult to express, since the mechanisms behind amyloidosis development are partly unknown. However, 80% Dodecylbenzene seems to be generally somewhat toxic to the animals.

Skin toxicity

As to skin toxicity, the most pronounced hyperplasia and some ulcerations were seen after 80% Dodecylbenzene and after DMBA followed by 40% Dodecylbenzene. So it can be concluded that there is some skin toxicity for hairless mice exerted by Dodecylbenzene.

FINAL CONCLUSION

It can be concluded that Dodecylbenzene has a weak skin tumorigenicity on mice, both as a complete carcinogen and as regards enhancement of DMBA carcinogenesis. There may also be a weak effect on the lymphoid system, but this is not really significant.

It is difficult to say whether such results in animals are of significance for the human situation. It is traditional to accept tumorigenicity in animals as a sign of possible carcinogenic hazard for humans. If the result of this test should be of any significance, one would say that Dodecylbenzene may be a weak, complete carcinogen and a weak promoter. Since humans are usually more resistent than mice, and the latency time is much longer, one might venture the opinion that the hazard is probably small and of little practical significance. Generally, good hygiene at the working place with reduction of exposure as much as possible, seems to me to be the only necessary precautions.

Oslo, 23rd September 1985

Olav Hilmar Iversen
Professor of Pathology

TABLE 1 - CARCINOGENICITY OF DODECYLBENZENE - TUMOR ANALYSIS AND PATHOLOGICAL FINDINGS

Group no.:	1	2	3	4	6
Treatment:	Acetone	Dodecylbenzene 16%	Dodecylbenzene 80%	DMBA alone	DMBA + 40% dodecylbenz.
Type of tumor:					
Skin tumors:					
Papillomas	3/2	4/4	13/9	56/27	68/26
Carcinomas	0	1/1	1/1	6/6	6/6
Sarcomas	0	1/1	0	0	7/7
Sum skin tumors:	3/2	6/6	14/9	62/27	81/26
Lymphoid tumors:					
B-cell lymphoma	0	0	0	1/1	0
Lymphoma NOS	2/2	2/2	6/6	4/4	4/4
Sum lymphomas:	2/2	2/2	6/6	5/5	4/4
Reticuloses	3/3	4/4	1/1	2/2	2/2
Sum lymphoid lesions:	5/5	6/6	7/7	7/7	6/6
Lung adenomas:	4/3	8/6	6/6	16/12	7/5
Per cent of autopsied	10	20	11	27	13
Other tumors:					
Abdominal sarcoma	1/1	1/1	0	0	1/1
Angiosarcoma of liver	0	0	1/1	0	1/1
Hepatoma	0	0	1/1	0	0
Amyloidosis:	2/2	9/9	22/22	14/14	6/6
Per cent of autopsied	5	23	39	23	11
Skin toxicity:					
Pronounced hyperplasia	0	+	++	0	++
Ulcerations	0	0	+	0	+

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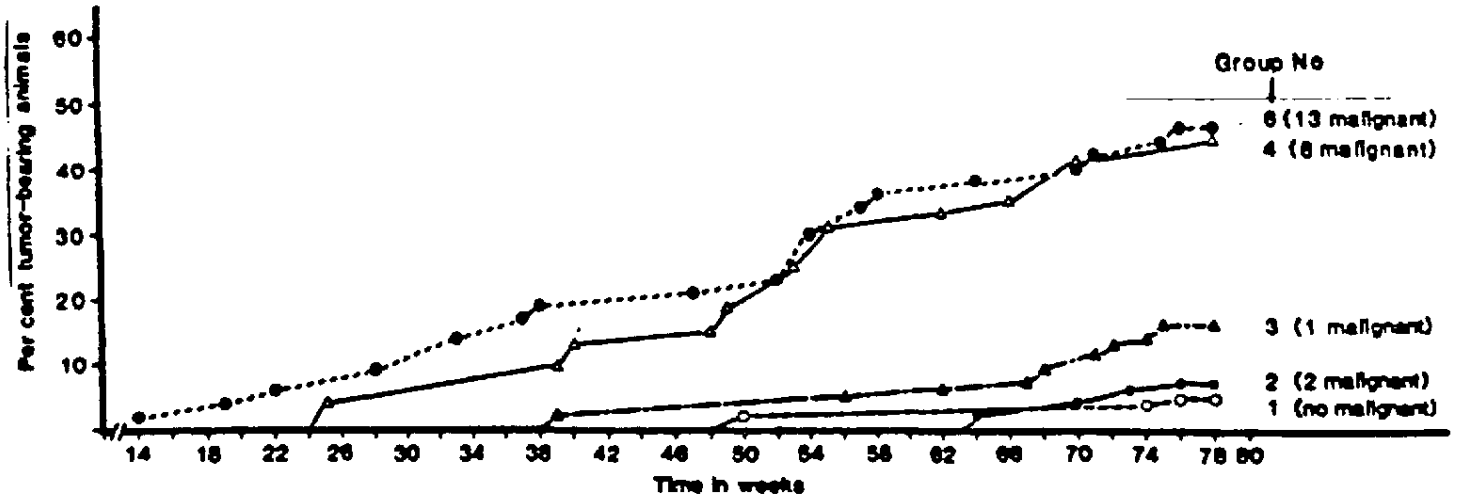
Fig. 1. Skin carcinogenicity of Dodecylbenzene - tumor rates (% tumor-bearing animals) versus time



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ig. 2. Skin carcinogenicity of Dodecylbenzene
- tumor yields (total number of tumors)
versus time

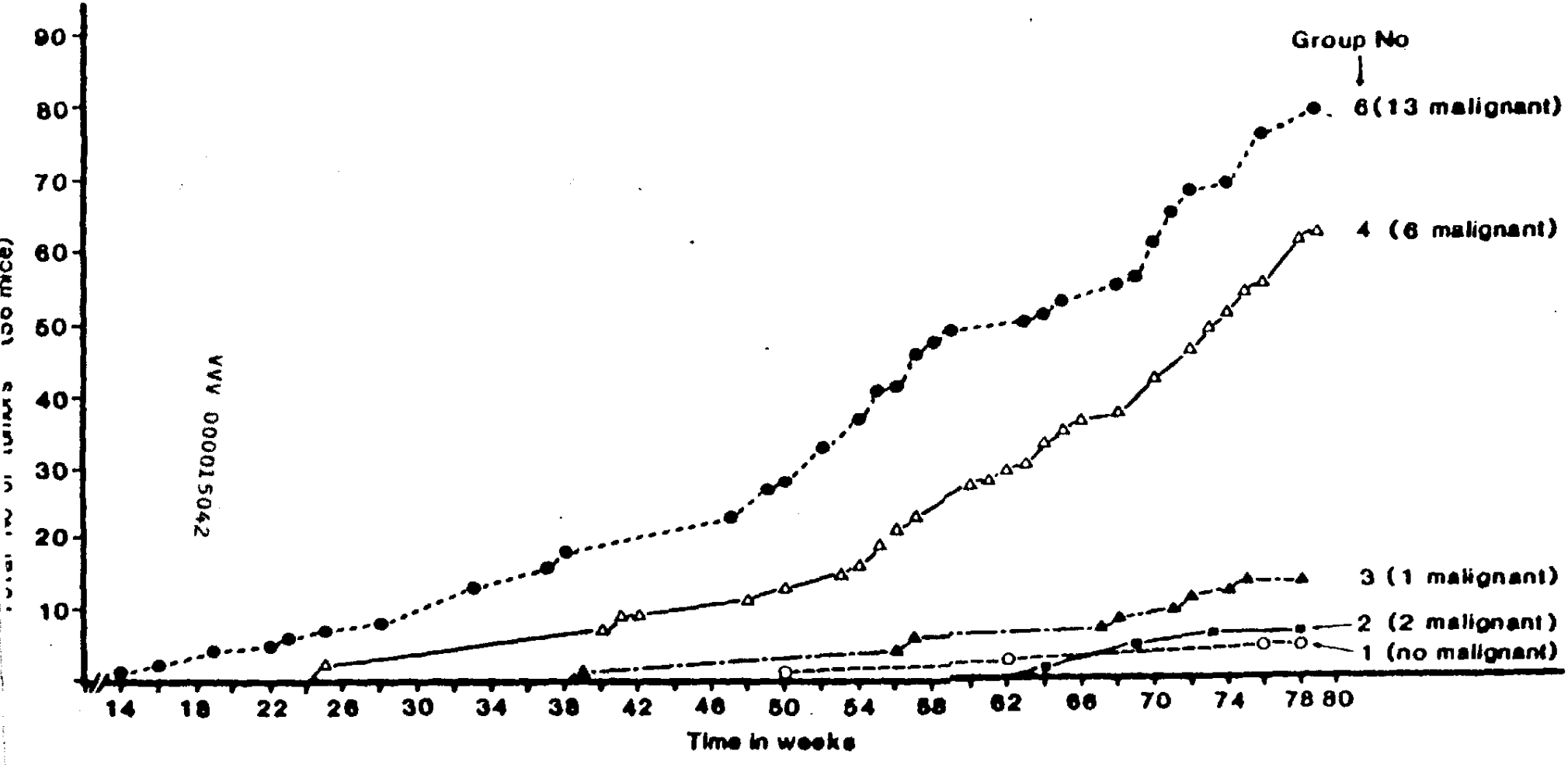

TABLE 2 - SKIN CARCINOGENICITY OF DODECYLBENZENE - STATISTICAL EVALUATION

	Tumor rates						Tumor yields				
	Overall obs/exp	1 v 3 obs/exp	1 v 4 obs/exp	1 v 5 obs/exp	6 v 4 obs/exp	6 v 5 obs/exp	1 v 3 odds	1 v 4 odds	1 v 5 odds	6 v 4 odds	6 v 5 odds
1. Acetone alone	0.08	0.26	0.08	0.06			1.00	0.03	0.04		
2. 16% Dodecyl-benzene alone	0.30										
3. 80% Dodecyl-benzene alone	0.58	1.94					8.69 (week 57)				
4 51.2 ug DMBA once (this study)	2.27		2.39		1.12			1.00		1.56 (week 38)	
5 51.2 ug DMBA once (128 mice)	-			1.49		1.37			1.00		1.55 (week 39)
6. 51.2 ug DMBA one and 40% dode-cylbenzene	2.85				0.89	0.89				1.00	1.00
T	39.01	2.91	11.64	15.70	2.43	6 18					
One-tailed P value for positive trend	0.0001	0.0130	<0.0001	<0.0001	0 2274	0.0406					
Chi square	64.40	4.96	29.26	23 97	0.56	3.04	5 45	28.82	25 22	3.34	4.52
Degree of freedom	4	1	1	1	1	1	1	1	1	1	1
P value for heterogeneity	<0.0001	0.0260	<0.0001	<0.0001	0.4547	0.0811					
Best P value for tumor yield							0.025>P>0.010	<0 0005	<0.0005	0 10>P>0.05	0.05>P>0 025
Conclusion	v.s	s.	v.s.	v.s.	n.s.	sugg.	s.	v.s.	v.s.	sugg.	s.

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