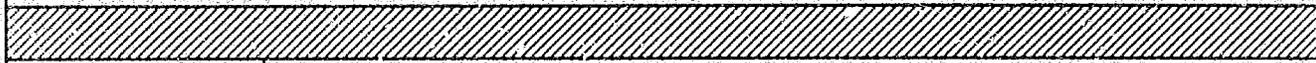


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INTRA-LABORATORY CORRESPONDENCE

"OAK RIDGE NATIONAL LABORATORY"

August 21, 1984

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TO: George Dorsey
FROM: Mike Holland-- *upjohn, Kolanszko*
SUBJECT: Preliminary Findings for Y12-III

NOV 21 1986

CONTAINS NO CB

This letter summarizes the salient findings of chronic dermal toxicity and skin tumorigenicity following two-year, thrice-weekly topical application of four test articles in appropriate solvents.

Solvent stability studies were conducted to determine an appropriate vehicle for dilution of each test article. The final choice was the optimum on the basis of chemical stability, solubility and biological inertness. The materials are identified in Table 1 together with the respective concentrations in the corresponding solvent.

Body weight was determined at 6, 12, 18 and 24 months of exposure at the highest dose of each material. All animals, whether dying spontaneously, killed due to the presence of a skin neoplasm or at the end of the study were subjected to a complete gross necropsy with examination of all abdominal and thoracic viscera. Gross lesions were recorded and, where uncertainty existed, samples were taken for histologic evaluation. All skin tumors were verified and classified histologically.

A statistically significant incidence of skin neoplasms was not induced by any of the test materials after two years of continuous exposure (Table 2). There were interesting differences between solvents in the pattern and characteristics of squamous tumors observed in positive controls. Benzo(a)pyrene applied in methanol appeared to induce higher grade neoplasms than an equivalent concentration applied in cyclohexane. There also appeared to be an interaction between exposure rate and histologic grade of squamous tumors; tumors induced at high dose rate tended to be more histologically malignant than those induced at lower dosage rates.

Overall mortality patterns varied for each material. For nos. 4 and 5, no consistent pattern was observed. However, mortality significantly greater than corresponding vehicle control was noted in mid-dose females exposed to no. 5 and mid-dose males exposed to no. 6 (Table 3A). I do not consider these differences as indicative of compound related toxicity since there is no clear dose response. For nos. 2 and 3, the situation was much different. Significant and dose dependent increases in mortality were observed for both materials in both sexes (Table 3B). Both materials are systemically toxic via the dermal route.

Reinforcing the mortality data are body weight trends summarized in Table 4. While these data have not yet been evaluated statistically there

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appears to be a positive correlation between depressed body weight gain and increased mortality. However, body weight depression was also noted for solvent control animals versus untreated room controls suggesting the solvents themselves are not totally innocuous.

Upon death or scheduled sacrifice each animal was necropsied and selected organs were weighed. Tables 5A and 5B summarize the incidence of grossly observed neoplasms in a set of major organs. For nos. 2 and 3 (5A), there appeared to be a treatment-related increase in liver tumor incidence for both materials in both sexes. The data represent crude incidences and in view of known dose-dependent differences in overall mortality a more accurate assessment of tumor risk versus exposure rate will require an actuarial statistical analysis. For this summary the crude incidence in females is a good measure of the activity of the two materials compared to the solvent controls. On this basis it would appear that no. 3 is more active than 2 and that a no-effect level has not been demonstrated for either material. For the remaining organs there appeared to be no obvious increase or decrease in tumor incidence correlating with exposure and dose. For nos. 4 and 5 (Table 5B), no pattern of increase or decrease in tumor incidence was apparent. However, the slight increase in liver tumor incidence noted in males treated with no. 4 may be significant but a definite conclusion must await appropriate statistical analysis.

Based upon the data available at the time of this summary it is my conclusion that none of the compounds evaluated pose a skin carcinogenic hazard. Materials 2 and 3 both show the potential to induce systemic toxicity associated with long term dermal exposure and are capable of inducing or enhancing development of hepatocellular neoplasms in the male and female C3H mouse. Treatment-related grossly observable neoplasms at other sites, including the thyroid, were not detected.

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Enclosures

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Table 1. Experimental Design

Compound	CAS No.	Test ID	Solvent	Concentration, w/v%	Dosage, mg/uk [†]
m-Phenylenediamine					
Methylenedianiline	Mixture	2	Methanol	6,3,1.5	10.8,5.4,2.7
Diglycidylether of bisphenol-A					
Methylenedianiline	101-77-9	3	Methanol	3,1.5,0.75	4.5,2.25,1.12
γ Glycidyloxy-trimethoxysilane	2530-83	4	Cyclohexane	50,40,30	78,62.4,46.8
γ Aminopropyl-triethoxysilane	919-30	5	Cyclohexane	30,20,10	43.8,29.2,14.6

[†]Corrected for density, based upon three applications per week Monday, Wednesday, and Friday in 50 ul of the solvent.

Material (solvent)	Exposure rate (mg/wk)	No. of Histologically Confirmed Skin Tumors (sexes combined)			
		Papilloma	Squamous Carcinoma	Fibroma	Other
2 ^a	10.8	0	0	0	0
(Methanol)	5.4	0	0	0	0
	2.7	0	0	0	0
3 ^a	4.5	0	0	0	0
(Methanol)	2.25	0	0	0	0
	1.12	0	0	0	0
4 ^a	78.0	0	0	0	0
(Cyclohexane)	62.4	0	0	0	0
	46.8	0	0	0	0
5 ^a	43.8	2	0	0	0
(Cyclohexane)	29.2	0	0	0	0
	14.6	0	0	0	0
B(a)P	0.015	11	60	0	0
(Cyclohexane)	0.0075	14	43	1	1
	0.00375	28	19	1	2
B(a)P ^a	0.015	3	75	0	0
(Methanol)	0.0075	11	62	0	0
	0.00375	10	23	4	0
Cyclohexane ^b	116.7	1	0	0	0
Methanol ^b	119	0	0	0	0

^a40 animals per sex, per dose.

^b100 animals per sex, per dose.

Table 3A. Mortality Analysis for Materials Applied in Cyclohexane

Material	Sex	Dose (mg/wk)	24 Month survival (%)	Risk Coefficients [†]		
				$\hat{\beta}$	sd	$\frac{\hat{\beta}}{sd}$ ^{††}
Cyclohexane	F	116.7	49.4	-	-	-
	M	116.7	60.2	-	-	-
4	F	78	48.7	0.30	0.24	
		62.4	32.5	0.55	0.23	2.4
		46.8	52.6	0.01	0.26	
	M	78	51.3	0.14	0.26	
		62.4	51.3	0.08	0.27	
		46.8	47.5	0.16	0.26	
5	F	43.8	50.0	0.12	0.25	
		29.2	48.6	0.03	0.26	
		14.6	61.5	-0.06	0.26	
	M	43.8	48.6	0.30	0.26	
		29.2	40.5	0.52	0.25	2.1
		14.6	50.0	0.11	0.26	

[†]From the proportional hazards regression model $\lambda_j(t) = \lambda_0(t)e^{\beta_j}$, where $\lambda_0(t)$ denotes the "force of mortality" at age t for the vehicle control group so that e^{β_j} ($j =$ dose group A,B,C) is the "relative risk" for dose group j . β is positive if the risk in treated animals is greater, 0 if equal and negative if less than control.

^{††}For $\frac{\hat{\beta}}{sd} > 1.95$ $P \leq 0.05$. Values are shown only if $P \leq 0.05$.

Table 3B. Mortality Analysis for Materials Applied in Methanol

Material	Sex	Dose (mg/wk)	24 Month survival (%)	Risk Coefficients [†]		
				$\hat{\beta}$	sd	$\frac{\hat{\beta}}{sd}$ ^{††}
Methanol	F	119	60.7	-	-	-
	M	119	62.9	-	-	-
2	F	10.8	20.5	1.14	0.24	4.7
		5.4	53.8	0.27	0.27	
		2.7	61.5	0.02	0.28	
	M	10.8	32.5	0.78	0.24	3.2
		5.4	35.0	0.74	0.24	3.1
		2.7	37.5	0.53	0.25	2.1
3	F	4.5	15.4	1.39	0.24	5.8
		2.25	25.7	0.97	0.25	3.9
		1.12	35.9	0.82	0.25	3.3
	M	4.5	35.0	0.83	0.24	3.5
		2.25	35.9	0.66	0.25	2.6
		1.12	62.5	-0.12	0.29	

[†]See Table 3A.

^{††}See Table 3A.

Table 4. Body Weight Trends

Material (solvent)	Sex	Dose (w/v%)	Average Weight (\pm se), g			
			6 Months	12 Months	18 Months	24 Months
2 (Methanol)	F	6%	26.7(0.4)	28.8(0.5)	29.7(0.6)	25.4(0.7)
	M		33.8(0.3)	34.4(0.4)	34.1(0.5)	35.1(0.6)
3 (Methanol)	F	3%	29.5(0.3)	30.8(0.4)	32.5(0.5)	28.2(1.0)
	M		35.2(0.4)	36.6(0.5)	35.0(0.6)	26.7(0.7)
4 (Cyclohexane)	F	50%	27.9(0.3)	28.5(0.4)	30.4(0.5)	27.2(0.7)
	M		33.4(0.3)	34.4(0.4)	34.6(0.5)	28.9(0.8)
5 (Cyclohexane)	F	30%	28.5(0.3)	29.9(0.3)	31.0(0.4)	27.9(0.6)
	M		34.3(0.6)	35.9(0.7)	36.1(0.6)	28.2(0.9)
B(a)P (Cyclohexane)	F	0.01%	29.4(0.3)	31.1(0.4)	N.D.	29.5(0.3)
	M		34.4(0.3)	36.5(0.4)	N.D.	34.4(0.3)
(Cyclohexane)	F	100%	28.9(0.3)	30.2(0.4)	31.0(0.5)	26.9(0.5)
	M		33.5(0.5)	35.9(0.5)	34.6(0.6)	27.6(0.4)
B(a)P (Methanol)	F	0.01%	29.7(0.4)	31.0(0.4)	N.D.	29.1(0.4)
	M		35.3(0.4)	35.5(0.4)	N.D.	34.0(0.5)
(Methanol)	F	100%	28.8(0.4)	29.9(0.5)	29.6(0.7)	27.4(0.4)
	M		36.3(0.5)	37.5(0.6)	37.1(0.6)	29.3(0.5)
Untreated	F	-	N.D.	N.D.	33.1(0.8)	30.1(0.9)
Untreated	M	-	N.D.	N.D.	44.3(0.6)	34.4(1.8)

Table 5A. Frequency of Grossly Observed Neoplasms for Materials Applied in Methanol

Material	Sex	Dose (mg/wk)	Observed Tumor Incidence (%)				
			Liver	Spleen	Kidney	Ovary/Testis	Lung
2 [†]	F	10.8	25	0	4	40	0
		5.4	25	0	0	55	8
		2.7	20	0	0	68	10
	M	10.8	55	0	0	0	0
		5.4	68	0	0	0	2
		2.7	60	0	0	0	5
3 [†]	F	4.5	85	2	4	52	10
		2.25	25	0	0	40	10
		1.12	22	0	2	55	8
	M	4.5	95	0	2	0	12
		2.25	75	0	0	0	2
		1.12	55	0	0	0	8
Methanol ^{††}	F	119	11	2	2	49	16
	M	119	53	0	0	0	9

[†]40 animals per sex, per dose.

^{††}100 animals per sex.

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Table 5B. Frequency of Grossly Observed Neoplasms for Materials Applied in Cyclohexane

Material	Sex	Dose (mg/wk)	Observed Tumor Incidence (%)				
			Liver	Spleen	Kidney	Ovary/Testis	Lung
4 [†]	F	78	12	0	2	60	15
		62.4	18	0	0	52	2
		46.8	12	0	0	52	15
	M	78	62	0	2	0	8
		62.4	60	0	2	2	12
		46.8	42	0	2	0	2
5 [†]	F	43.8	12	0	0	45	8
		29.2	12	0	0	50	12
		14.6	18	0	4	50	12
	M	43.8	48	0	0	2	15
		29.2	58	0	2	0	15
		14.6	58	0	0	0	18
Cyclohexane ^{††}	F	117	18	0	3	44	10
	M	117	52	0	1	0	13

[†]40 animals per sex, per dose.

^{††}100 animals per sex, per dose.