



The mean body weights of dosed males were 3% to 14% lower than those of vehicle controls from week five to the end of the study. The mean body weights of high dose females were 5% to 17% lower than controls from week five to the end of the study. Mean body weights of low dose females and control females were similar. No compound related clinical signs were seen in any dose group.

Despite the absence of clinical signs and only small changes in mortality, 8-MOP caused significant microscopic damage, particularly in males. The most severe damage was in the kidneys. 8-MOP caused a spectrum of degenerative and proliferative changes in the kidneys of male rats. Nephropathy occurred in nearly all male rats including controls but the severity and extent of the damage was significantly greater in dosed rats. The nephropathy included degeneration and regeneration of the tubular epithelium with dialation and atrophy of the tubules, formation of hyaline and granular casts, thickening of the basement membranes, interstitial fibrosis and glomerulosclerosis. Dosing caused focal hyperplasia of the renal tubular epithelium and focal enlargement of individual tubules. Frequently, the epithelium was stratified and the cells showed loss of basement membrane dependency. There also was mineralization of the renal papilla. Dosing also caused hyperplasia, adenomas and adenocarcinomas of the tubular epithelium. The following table shows the increased incidence of nephropathy and proliferative disease in the kidney.

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Renal Lesions in Male Rats	vehicle Control	%	37.5 mg/kg	%	75 mg/kg	%
Mineralization of the Renal Papilla	0/50	0	0/50	0	31/49	63
Focal Hyperplasia of Renal Tubules	0/50	0	8/50	16	8/49	16
Nephropathy						
Overall Rate	48/50	96	49/50	98	47/49	96
Grade 1 (minimal)	10/50	20	10/50	20	7/49	14
Grade 2 (mid)	28/50	56	13/50	26	9/49	18
Grade 3 (moderate)	9/50	18	13/50	26	16/49	33
Grade 4 (marked)	1/50	2	13/50	26	15/49	31
Tubular Cell Adenoma						
Overall Rate	1/50	2	11/50	22	8/49	16
Adjusted Rate	3.3%		45		30.5	
Terminal Rate	1/30	3	4/16	25	2/16	13
Week of first observation	106		95		80	
Life Table Test, P value	0.003		<0.001		0.004	
Incidental Tumor Test, P value	0.031		0.004		0.026	
Logistic Regression Analysis, P value	0.008		<0.001		0.009	
Tubular Cell Adenocarcinoma						
Overall Rate	0/50	0	1/50	2	3/49	6
Adjusted Rate	0		6.2		15.2	
Terminal Rate	0/30	0	1/16	6	2/16	13
Week of first observation			105		92	
Life Table Test, P value	0.024		0.375		0.53	
Incidental Tumor Test, P value	0.024		0.375		0.55	
Logistic Regression Analysis, P value	0.024		0.375		0.78	
Tubular Cell Adenoma or Adenocarcinoma						
Overall Rate	1/50	2	12/50	24	11/49	22
Adjusted Rate	3.3		49.6		42.3	
Terminal Rate	1/30	3	5/16	31	4/16	25
Week of first observation	106		95		80	
Life Table Test, P value	0.001		<0.001		<0.001	
Incidental Tumor Test, P value	0.003		0.001		0.002	
Logistic Regression Analysis, P value	0.001		<0.001		0.001	

Dosing increased the incidence of parathyroid hyperplasia in dosed males (vehicle 2/49, low dose 22/47, high dose 18/48). The incidence of fibrous osteodystrophy of the bone increased with dosing (vehicle 2/50, low dose 10/50, high dose 12/50). This pathology was considered secondary to the renal disease and renal secondary hyperparathyroidism.

The incidences of carcinoma or squamous cell carcinoma of the Zymbal gland was increased in males (vehicle 1/50, low dose 7/50, high dose 4/49). This increase was statistically significant. These neoplasms consisted of interconnecting masses or cords of stratified epithelial cells with glandular or squamous differentiation. They invaded the adjacent connective tissue.

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Zymbal Gland Neoplasia	vehicle Control	%	37.5 mg/kg	%	75 mg/kg	%
Carcinoma or Squamous Cell Carcinoma						
Overall Rate	1/50	2	7/50	14	4/49	8
Adjusted Rate	3.3		29.1		13.1	
Terminal Rate	1/30	3	2/16	13	0/16	0
Week of first observation	106		83		78	
Life Table Test, P value	0.063		0.008		0.104	
Incidental Tumor Test, P value	0.229		0.051		0.233	
Logistic Regression Analysis, P value	0.125		0.018		0.16	

Dosing caused a statistically significant increase in subcutaneous fibroma in male rats (vehicle 1/50, low dose 5/50, high dose 7/49). One rat in the high dose group developed a subcutaneous sarcoma.

Fibroma or Sarcoma in Male Rats	vehicle Control	%	37.5 mg/kg	%	75 mg/kg	%
Overall Rate	1/50	2	5/50	10	8/49	16
Adjusted Rate	3.3		24.1		32.3	
Terminal Rate	1/30	3	2/16	13	4/16	25
Week of first observation	106		100		13	
Life Table Test, P value	0.002		0.029		0.003	
Incidental Tumor Test, P value	0.009		0.0115		0.008	
Logistic Regression Analysis, P value	0.011		0.040		0.024	

Alveolar epithelial hyperplasia occurred at a greater incidence in dosed males (vehicle 5/50, low dose 7/50 and high dose 9/49). This hyperplasia was characterized by alveoli lined with increased numbers of cuboidal or columnar epithelial cells. Increased numbers of cells often distorted the alveolar structure. Alveolar or bronchiolar adenomas occurred in 4 of 50 vehicle control males, 9 of 50 low dose males, and 9 of 49 high dose males. The following table demonstrates this statistically significant increase.

Alveolar or Bronchiolar Adenomas in Male Rats	vehicle Control	%	37.5 mg/kg	%	75 mg/kg	%
Overall Rate	4/50	8	9/50	18	9/49	18
Adjusted Rate	12		37.4		35.8	
Terminal Rate	3/30	10	4/16	25	3/16	19
Week of first observation	84		87		68	
Life Table Test, P value	0.015		0.022		0.022	
Incidental Tumor Test, P value	0.05		0.077		0.131	
Logistic Regression Analysis, P value	0.048		0.075		0.069	

Dosed female rats had squamous cell papillomas of the oral cavity (vehicle 0/50, low dose 1/50, high dose 3/50). This incidence is probably significant when compared to historical controls.

Dosing caused diffuse hypertrophy of the thyroid gland in male rats (vehicle 2/50, low dose 31/50, high dose 39/49). Follicular cell adenomas or carcinomas (combined) were seen in 1 of 50 controls, 3 of 50 low dose males and 3 of 49 high dose males. The hypertrophy is possibly dose related but the neoplasm is a normal finding in mature male rats.

An increased number of cysts in the preputial gland were observed in high dose male rats (vehicle 6/50, low dose 4/50 and high dose 20 of 49). The incidence of adenomas of the anterior

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pituitary actually decreased in male and female rats (in males, vehicle 24/49, low dose 12/50, high dose 12/48; in females, vehicle 24/49, low dose 24/49 and high dose 15 of 49). Dosing caused chronic inflammation, ulcers, and epithelial hyperplasia in males. Two low dose male rats (4%) developed squamous cell papillomas. This is considerably greater than the historical control rate of 0.4%.

Interstitial cell tumors were increased in the testes of dosed males. Though the background incidence was high (vehicle 38/50, low dose 44/50, high dose 43/49) the increase showed a statistically significant positive trend.

In the eye, hemorrhage occurred in 1 of 50 control males, 14 of 50 low dose males and 9 of 49 high dose males. A high incidence of cataracts occurred in all groups, including controls and in both males and females, but this toxicity may have been related to the fluorescent lighting. Nevertheless, light activates 8-MOP to a radical and dosing may exacerbate the formation of cataracts.

Discussion:

Toxicity and hyperplastic changes in the kidneys of males were the most pronounced adverse effects associated with 8-MOP dosing. This toxicity did not occur in females. This suggests that there is a difference in metabolism between the two sexes. Such is common in rats. It is possible that the damage is due to a metabolite formed only by males. 8-MOP is extensively metabolized. Increased incidence of kidney damage or hyaline droplet formation and associated changes were not seen in the kidneys of rats dosed for 13 weeks (above).

The renal disease may cause a decrease in serum calcium. Thus, the pathology seen in the parathyroid may be due to compensation. This compensatory response is seen with other kidney toxins. Hyperplasia results from the prolonged stimulation of parathyroid hormone secretion by the low calcium concentrations. The fibrous osteodystrophy of the bone also may occur secondary to the kidney damage and the resulting derangement of calcium homeostasis.

8-MOP also caused tumors in the Zymbal gland. The incidence of these tumors was well above the NTP historical controls (0.8 %). The incidence of fibromas was also significantly above historical controls (9 %).

Perhaps the most troubling tumors were those seen in the lungs. Again the incidence was above historical controls (3%) and the tumors were almost certainly drug related. These tumors possibly form because of a difference between pulmonary and hepatic metabolism.

8-MOP has been shown to intercalate with DNA. It is mutagenic in vitro and in vivo (below). So it comes as no surprise that relatively large doses over a lifetime are carcinogenic to rats. The official statement of the NTP is that there is "clear evidence of carcinogenic activity" with 8-MOP.

Carcinogenicity Summary:

8-MOP in the absence of light is carcinogenic in rats. Rats dosed with up to 75 mg/kg (450 mg/m²) five days per week for 103 weeks developed a broad spectrum of dose related tumors. This increase occurred predominantly in males. Dosing also caused an increase in the incidence of hyperplasia, adenomas and adenocarcinomas of the tubular epithelium of the kidneys. Dosing increased the incidence of parathyroid hyperplasia in dosed males. The incidence of fibrous osteodystrophy of the bone also increased. This pathology was probably secondary to the renal toxicity and the resulting hyperparathyroidism. The incidences of carcinoma or squamous cell carcinoma of the Zymbal gland

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was increased in males. Alveolar epithelial hyperplasia occurred at a greater incidence accompanied by an increase in alveolar or bronchiolar adenomas in males. Dosed female rats had squamous cell papillomas of the oral cavity. Dosing caused diffuse hypertrophy of the thyroid gland in male rats. Follicular cell adenomas or carcinomas (combined) were increased. An increased number of cysts in the preputial gland were observed in high dose male rats. Dosing caused chronic inflammation, ulcers, and epithelial hyperplasia in the stomachs of males. Lastly, interstitial cell tumors were increased in the testes of dosed males. The doses in this study were many times higher than the proposed dose in the current NDA.

In a prospective study of 1373 patients given oral 8-methoxypsoralen photochemotherapy for psoriasis, thirty patients developed a total of 48 basal-cell or squamous-cell carcinomas. The observed incidence of cutaneous carcinoma was 2.63 times that expected for an age, sex and geographically matched population. Patients with a previous cutaneous carcinoma had a relative risk of 10.22. New psoriasis patients with histories of ionizing radiation exposure or previous skin tumor should be informed of this increased risk of skin cancer.

Genetic Toxicology:

- 1) NTP Technical Report on the Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen in F344/N Rats (gavage studies). NIH Publication number 89-2814. Ames Test. Volume 8, page 58.

Species	<i>Salmonella typhimurium</i>
Strains	TA98, TA100, TA102, TA104, TA1535, in triplicates with repetition
Drug	8-MOP
Light conditions	Yellow light, to prevent UVA activation of 8-MOP
Metabolic Activation	Aroclor 1254 induced male Sprague-Dawley rat or Syrian hamster liver
Incubation	20 minutes before the addition of soft agar Then a further 48 hours
Positive control	Without S9 – 4-nitro-o-phenylenediamine with TA98 Without S9 – sodium axide with TA100, TA1535 Without S9 – mitomycin C with TA102 and TA104 With S9 – 2-aminoanthracene with TA100, TA1535 and TA98 With S9 – sterigmatocystin with TA102 and TA104
Negative control	Solvent – DMSO

This was a GLP Study.

In the absence of S9 activation, 8-MOP was a weakly positive mutagen with strain TA104. It was negative with the other strains.

With S9 activation (5% or 30%), 8-MOP was a mutagen with strain TA102. This was evidenced by an approximately two-fold increase in revertants over control. With 10% S9 it was weakly positive (increase less than two fold but statistically significant) or equivocal in this strain. In Strain TA104 it was positive at all concentrations of hamster or rat S9. In strain TA100 8-MOP was a mutagen with all concentrations of hamster S9, but was positive only at the highest concentration of rat S9. 8-MOP was not mutagenic to TA1535 with or without activation. It was weakly positive with TA98 in the presence of 30% rat S9. The following tables show the results of the experiments in TA102, TA104 and TA100.

TABLE 19. MUTAGENICITY OF 8-METHOXYPSORALEN IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose ($\mu\text{g}/\text{plate}$)	Revertants/Plate (b)						
		-S9	+S9 (hamster)			+S9 (rat)		
			5%	10%	30%	5%	10%	30%
TA102	0	284 \pm 15.0	450 \pm 10.6	622 \pm 40.2	357 \pm 21.9	405 \pm 13.5	489 \pm 11.1	373 \pm 39.0
	0.3	--	--	--	468 \pm 6.4	--	--	327 \pm 14.3
	1	--	--	--	445 \pm 3.2	--	--	341 \pm 23.1
	3	--	512 \pm 6.0	665 \pm 5.5	427 \pm 20.3	--	--	315 \pm 13.6
	10	313 \pm 7.0	517 \pm 16.7	623 \pm 31.0	427 \pm 14.6	478 \pm 12.7	585 \pm 24.8	352 \pm 22.2
	33	304 \pm 2.6	592 \pm 22.5	687 \pm 34.0	453 \pm 33.7	530 \pm 6.4	601 \pm 8.8	406 \pm 31.1
	66	237 \pm 13.3	--	--	575 \pm 3.2	510 \pm 14.0	595 \pm 19.4	522 \pm 11.7
	100	307 \pm 15.6	744 \pm 35.2	699 \pm 14.1	682 \pm 15.3	659 \pm 30.3	755 \pm 28.8	590 \pm 23.2
	166	283 \pm 6.7	1,002 \pm 28.4	820 \pm 30.4	653 \pm 27.9	831 \pm 25.8	595 \pm 73.1	716 \pm 42.8
Trial summary		Negative	Positive	Equivocal	Positive	Positive	Weakly positive	Positive
Positive control (c)		586 \pm 43.9	1,577 \pm 103.0	1,990 \pm 48.3	600 \pm 9.2	971 \pm 34.5	1,324 \pm 59.1	1,487 \pm 26.6
TA104	0	316 \pm 18.5	539 \pm 14.8	538 \pm 3.8	376 \pm 1.5	450 \pm 15.2	515 \pm 12.4	359 \pm 20.5
	0.3	--	--	--	414 \pm 8.5	--	--	369 \pm 7.0
	1	--	--	--	447 \pm 11.4	--	--	428 \pm 9.2
	3	--	605 \pm 8.0	633 \pm 28.6	457 \pm 4.7	--	--	424 \pm 17.5
	10	378 \pm 12.0	662 \pm 21.4	767 \pm 14.7	459 \pm 9.5	725 \pm 26.1	843 \pm 20.5	639 \pm 21.8
	33	424 \pm 16.0	834 \pm 35.2	842 \pm 54.5	522 \pm 19.5	865 \pm 20.6	974 \pm 24.9	615 \pm 12.0
	66	405 \pm 33.1	--	--	667 \pm 21.8	989 \pm 18.6	1,087 \pm 8.7	833 \pm 16.7
	100	474 \pm 42.4	976 \pm 46.4	909 \pm 26.6	769 \pm 3.5	1,061 \pm 23.2	1,206 \pm 31.7	972 \pm 9.3
	166	389 \pm 48.8	1,059 \pm 59.6	1,046 \pm 83.3	807 \pm 18.7	1,157 \pm 59.6	1,242 \pm 13.0	911 \pm 63.5
Trial summary		Weakly positive	Positive	Positive	Positive	Positive	Positive	Positive
Positive control (c)	(d)	308 \pm 9.5	Toxic	Toxic	661 \pm 26.4	690 \pm 35.5	827 \pm 16.0	808 \pm 34.2
TA100	0	137 \pm 4.9	133 \pm 6.6	122 \pm 6.6	95 \pm 3.5	128 \pm 11.5	137 \pm 10.9	131 \pm 13.9
	10	--	--	--	--	186 \pm 7.0	145 \pm 9.8	163 \pm 7.4
	16	123 \pm 6.6	--	--	--	--	--	--
	33	127 \pm 13.7	141 \pm 5.8	138 \pm 5.8	114 \pm 3.7	154 \pm 12.1	163 \pm 4.4	191 \pm 27.7
	66	145 \pm 9.0	--	--	--	134 \pm 9.3	167 \pm 10.3	213 \pm 4.8
	100	125 \pm 8.7	162 \pm 15.0	125 \pm 16.7	122 \pm 10.6	141 \pm 9.7	154 \pm 4.4	229 \pm 9.7
	166	120 \pm 8.1	--	--	--	--	--	--
	333	--	349 \pm 13.4	179 \pm 10.4	168 \pm 11.4	138 \pm 13.0	147 \pm 8.8	256 \pm 4.3
	666	--	363 \pm 3.3	284 \pm 13.5	184 \pm 14.7	--	--	--
	1,000	--	321 \pm 19.6	317 \pm 13.9	190 \pm 30.0	--	--	--
Trial summary		Negative	Positive	Positive	Positive	Equivocal	Negative	Positive
Positive control (c)		376 \pm 5.9	645 \pm 26.0	466 \pm 23.8	642 \pm 12.3	396 \pm 20.2	324 \pm 6.2	263 \pm 5.8

8-MOP is mutagenic to *Salmonella in vitro* in the presence of S9.

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2) NTP Technical Report on the Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen in F344/N Rats (gavage studies). NIH Publication number 89-2814. Chinese Hamster Ovary Cytogenetics Assay. Volume 8, page 58.

Cells	Chinese Hamster Ovary (CHO)
Drug	8-MOP
Light conditions	"Gold" lights
Metabolic Activation	Aroclor 1254 induced Sprague-Dawley rat liver S9 fraction
Incubation SCE	26 hours with chemical with S9 8 hours with chemical without S9
Incubation CA.	2 hours with compound, 24 to 26 hours with BrdUr
Positive control	Mitomycin C without S9 Cyclophosphamide with S9
Negative control	Solvent - DMSO

SRI Laboratories did this study under GLP conditions.

In the absence of S9, 8-MOP caused a three-fold increase in the number of Sister Chromatid Exchange (SCEs) at a dose of 33.3 $\mu\text{g}/\text{ml}$. Other SCE parameters increased concomitantly and the results were repeated in a second trial at slightly different concentrations. In the presence of S9, 8-MOP caused a greater than two-fold increase in the number of SCEs and concomitant parameters at higher concentrations. S9 appeared to afford slight protection in this assay, probably secondary to microsomal metabolism. Nevertheless, 8-MOP remained clastogenic. The drug precipitated at concentrations above 333 $\mu\text{g}/\text{ml}$. The following table shows the results of the of the SCE assay experiments.

TABLE 20. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY 8-METHOXYPSORALEN (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/Chromosome	SCEs/Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
-S9 (c)								
Trial 1-Summary: Positive								
Dimethyl sulfoxide		50	1,046	413	0.39	8.3	25.7	-
8-Methoxypsoralen	3.3	50	1,048	593	0.57	11.9	25.7	143.4
	10	50	1,034	581	0.56	11.6	25.7	139.8
	33.3	50	1,028	1,207	1.17	24.1	25.7	290.4
	100	50	189	259	1.37	28.8	25.7	347.0
	333.3	0						
Mitomycin C	0.001	50	1,046	877	0.84	17.5	25.7	210.8
	0.01	5	106	230	2.17	46.0	25.7	554.2
Trial 2-Summary: Positive								
Dimethyl sulfoxide		25	526	167	0.32	8.7	25.7	-
8-Methoxypsoralen	20.2	25	515	387	0.75	15.5	25.7	231.3
	50.5	25	515	410	0.80	16.4	25.7	244.8
	100.5	5	103	101	0.98	20.2	25.7	301.5
	150	0						
Mitomycin C	0.001	25	519	284	0.55	11.4	25.7	170.1
	0.01	5	104	198	1.90	39.6	25.7	591.0
+S9 (d)								
Summary: Positive								
Dimethyl sulfoxide		50	1,047	391	0.37	7.8	25.7	-
8-Methoxypsoralen	33.3	50	1,044	553	0.53	11.1	25.7	142.3
	100	50	1,047	611	0.58	12.2	25.7	156.4
	(e) 333.3	50	1,046	910	0.87	18.2	25.7	233.3
	1,000	0						
Cyclophosphamide	0.4	50	1,036	644	0.62	12.9	25.7	166.4
	3	5	104	180	1.73	36.0	25.7	461.5

In the absence of S9, 8-MOP also caused as much as a 30 fold increase in the number of chromosomal aberrations per cell and the proportion of cells with aberrations at a concentration of 200 µg/ml. This result was repeatable. In these experiments, S9 protected the cells from chromosomal aberrations; there was no significant increase in aberrations per cell even at 500 µg/ml. The following table shows these results.

TABLE 21. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY 8-METHOXYPsorALEN (a)

		Trial 1			Trial 2				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/Cell	Percent Cells with Abs
-S9 (b)-Harvest time 20.0 h (c)					-S9 (b)-Harvest time 20.2 h (c)				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	3	0.02	1.5		100	1	0.01	1.0
8-Methoxypsoralen					8-Methoxypsoralen				
100	200	21	0.11	9.5	200	100	19	0.19	15.0
150	200	22	0.11	10.5	225	100	15	0.15	15.0
200	100	42	0.42	35.0	250	100	33	0.33	29.0
Summary: Positive					Summary: Positive				
Mitomycin C					Mitomycin C				
0.05	200	39	0.20	14.5	0.05	100	34	0.34	26.0
0.08	25	20	0.80	52.0	0.08	25	29	1.16	52.0
+S9 (d)-Harvest time 12.0 h (c)					+S9 (d)-Harvest time 12.0 h (c)				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	5	0.03	2.5		100	2	0.02	2.0
8-Methoxypsoralen					8-Methoxypsoralen				
101	200	6	0.03	3.0	498	100	9	0.09	8.0
252	200	8	0.04	3.5	552	100	7	0.07	7.0
502.5	200	14	0.07	6.5	600	100	9	0.09	9.0
Summary: Negative					Summary: Negative				
Cyclophosphamide					Cyclophosphamide				
7.5	200	26	0.13	10.5	7.5	100	11	0.11	11.0
37.5	25	14	0.56	44.0	37.5	25	11	0.44	32.0

- 3) P Weniger. 1981. A comparison of the photochemical actions of 5 and 8-methoxypsoralen on CHO cells. Toxicology 22:53-58. Volume 10, page 258.

Dr. Weniger demonstrated that 8-MOP induced interstrand cross-links within the DNA of CHO cells after the cells were irradiated with UVA light. This treatment decreased the rate of DNA synthesis at sublethal doses of 8-MOP. The repair of damage caused by 8-MOP in combination with UVA light was not complete after 24 hours. 8-MOP did not cross link DNA in the absence of UVA light in these experiments.

Genotoxicity Summary:

8-MOP is mutagenic and clastogenic at concentrations as low as 15 μM in the absence of UV light. In the Ames test, in the absence of S9 activation, 8-MOP was a weakly positive mutagen with strain TA104. It was negative with the other strains. With S9, activation 8-MOP was a mutagen with strain TA102, TA104 and TA100. This was evidenced by at least a two-fold increase in mutations over control.

In the absence of S9, 8-MOP caused a three to four-fold increase in the number of Sister Chromatid Exchange (SCEs) in CHO cells at a dose of 33.3 $\mu\text{g}/\text{ml}$. In the presence of S9, 8-MOP caused a greater than two-fold increase in the number of SCEs and concomitant parameters at higher concentrations. S9 appeared to afford slight protection in this assay, probably secondary to microsomal metabolism.

In the absence of S9, 8-MOP also caused as much as a 30 fold increase in the number of chromosomal aberrations per cell and the proportion of cells with aberrations in CHO cells at a concentration of 200 $\mu\text{g}/\text{ml}$ (0.9 mM). In these experiments, S9 protected the cells from chromosomal aberrations. Numerous other experiments have shown that 8-MOP causes DNA damage, strand cross-links and errors in DNA repair. 8-MOP probably forms radicals in the absence of irradiation, but the genetic damage is increased in the presence of UV light.

Overall Summary:

After IV administration to rats, 8-MOP is rapidly and widely distributed. The volume of distribution is about 0.8 liters per kilogram. This value is independent of dose and blood concentration. The first order elimination half-life of small doses is about 0.24 hours. This value increases with dose as does the dose-normalized AUC. Clearance decreases with dose. This is because the elimination mechanisms, probably hepatic, for 8-MOP are saturable. In rats, saturation occurs above a serum concentration of 1 µg/ml or 5 µM, a relatively low concentration. The drug concentrations are relatively uniform in most major organs and usually just less than blood concentration. Higher concentrations are found in the liver, kidney and fat in rats. Similar results are seen in dog and humans. In monkeys, the increase in AUC with dose is also nonlinear.

Formulation or particle size in a suspension of oral doses can dramatically affect GI absorption and the resulting toxicological or pharmacokinetic parameters. Pharmacokinetic parameters measured after oral doses are highly variable.

Rats eliminate about 71% of the total radioactivity in the urine, about 25% in the feces. Dogs eliminate about 45% in the urine and 40% in the feces. Most 8-MOP metabolism appears to occur in the liver. Eleven different metabolites have been detected in blood and urine of rats. These include 8-hydroxypsoralen, 5-hydroxy-8-methoxypsoralen, 5,8-dihydroxypsoralen, 5,8-dioxopsoralen, 6-(7-hydroxy-8-methoxycoumaryl)-acetic acid. Thus, rats metabolize 8-MOP by O-demethylation, hydroxylation at the 5-position, hydrolysis of the lactone ring and oxidation of the furan ring. These pathways are similar to those identified in dogs and humans.

The single-dose LD₅₀ of 8-MOP in rats and mice is approximately 500 mg/kg. The IP LD₅₀ is about 200 mg/kg. These values are about the same in both species for both routes so the rat appears to be less sensitive to 8-MOP toxicity on a mg/m² basis. Values as high as 2 g/kg for the oral LD₅₀ have been reported. This apparent difference probably results when the particle size in the oral dosing suspension is large. This leads to poor absorption and higher apparent LD₅₀ values. Rodents dosed with toxic amounts of 8-MOP become hypoactive and ataxic. At higher dose levels, the animals suffer prostration, muscle twitches and convulsions. Most deaths occurred in the first few days after dosing. Evidently, the dose response curve is relatively steep.

Twelve oral doses of 400 mg/kg (2400 mg/m²) over 16 days killed one of five male rats and one of five female rats. Little or no toxicity occurred in lower dose groups. A dose of 400 mg/kg given five times a week for thirteen weeks killed six of ten males and eight of ten females. Compound-related microscopic changes included fatty changes in liver and adrenal glands, and atrophy in the testes, seminal vesicles, and prostate. The only clinical sign seen in this study was dose dependent decreased weight gain.

Rats dosed up to 75 mg/kg (450 mg/m²) five days a week for 103 weeks developed occult significant toxicity. This toxicity was more pronounced in males than in females. Despite the absence of clinical signs and only small changes in mortality, 8-MOP caused significant microscopic damage. The most severe dose dependent damage was in the kidneys. 8-MOP caused a spectrum of degenerative and proliferative changes in the kidneys of male rats. Nephropathy included degeneration and regeneration of the tubular epithelium with dilation and atrophy of the tubules, formation of hyaline and granular casts, thickening of the basement membranes, interstitial fibrosis and glomerulosclerosis and mineralization of the renal papilla.

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A dose of 18 mg/kg (216 mg/m²) given three times per week for 26 weeks rendered one of three female monkeys moribund on day 39. Three males survived this dose. The moribund animal showed signs of cellular congestion in the lungs, liver and kidney, involution of the thymus and multiple, globular calcifications of the gonads. This monkey also showed signs of hepatocellular degeneration and regeneration with Kupffer cell proliferation. Kupffer cell proliferation in the liver was the only sign of toxicity in surviving monkeys.

8-MOP is phototoxic. Ten mg/kg given before a 10 minute light dose (2 J/cm²) twice weekly for 10 months caused actinic changes in the skin of hairless mice. Doses of 20 mg/kg before a 10 minute light dose (2 J/cm²) twice weekly for eight months caused notable phototoxicity and chronic erythema. Higher doses of drug and light caused significant phototoxicity and extensive inflammatory changes with acute erythema, deep ulcers, and deformities. Doses of 20 mg/kg with a light dose of 10 minutes (2 J/cm²) six times a week for 3 months killed some of the mice. In some studies, this photo-damage leads to neoplastic changes. Doses that cause phototoxicity are about three times higher than the human dose for psoriasis on a mg/m² basis and about 500 times higher than the maximum doses possible in the current NDA.

Therakos has studied the photopheresis process extensively in dogs with the CENTRINET device. The maximum dose of extra-corporeal 8-MOP they studied was 500 ng/ml or about twice the normal dose used in photopheresis for humans. These studies showed that the photopheresis process causes little toxicity. The mild decreases in white cell parameters seen in some of these experiments result from the phototoxic mechanism of the process. The number of blood-draws required by the experimental protocols probably caused the mild decreases in red cell parameters.

Doses of 80, 120 and 160 mg/kg/d (480, 720, 960 mg/m²/d) during gestation caused significant fetal toxicity in rats. This toxicity was strongly associated with maternal weight loss, anorexia and increased relative liver weight. Thus, the fetal toxicity may be a direct consequence of maternal toxicity. Signs of fetal toxicity included increased fetal mortality, increased resorptions, late fetal death, fewer fetuses per litter and decreased fetal weight. Treatment caused an increase in skeletal malformation and variations. 8-MOP treatment is embryo-lethal and teratogenic, but only at doses greater than or equal to 80 mg/kg/day. Eighty mg/kg/day caused only minimal maternal toxicity. For psoriasis therapy, the average adult takes about 50 mg (30 mg/m²) of 8-MOP two hours before exposure to UVA light. So the dose that caused no maternal toxicity and only minimal fetal toxicity in this study, 80 mg/kg or 480 mg/m², is 16 times greater than the dose recommended for psoriasis therapy. This lowest fetotoxic dose is over 4000 times greater than the maximum re-infused dose in a single course of UVADEX therapy.

8-MOP is mutagenic and clastogenic at concentrations as low as 15 µM in the absence of UV light. In the Ames test, in the absence of S9 activation, 8-MOP was a weakly positive mutagen with strain TA104. It was negative with the other strains. With S9 activation 8-MOP a mutagen with strain TA102, TA104 and TA100. This was evidenced by at least a two-fold increase in mutations over control.

In the absence of S9, 8-MOP caused a three to four-fold increase in the number of Sister Chromatid Exchange (SCEs) in CHO cells at a dose of 33.3 µg/ml. In the presence of S9, 8-MOP caused a greater than two-fold increase in the number of SCEs and concomitant parameters at higher concentrations. S9 appeared to afford slight protection in this assay, probably secondary to microsomal metabolism.

In the absence of S9, 8-MOP also caused as much as a 30 fold increase in the number of chromosomal aberrations per cell and the proportion of cells with aberrations in CHO cells at a concentration of 200 µg/ml (0.9 mM). In these experiments, S9 protected the cells from chromosomal

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aberrations. Numerous other experiments have shown that 8-MOP causes DNA damage, strand cross-links and errors in DNA repair. 8-MOP probably forms radicals in the absence of irradiation, but the genetic damage is increased in the presence of UV light.

8-MOP in the absence of light is carcinogenic in rats. Rats dosed with up to 75 mg/kg (450 mg/m²) five days per week for 103 weeks developed a broad spectrum of dose related tumors. This increase occurred predominantly in males. Dosing also caused an increase in the incidence of hyperplasia, adenomas and adenocarcinomas of the tubular epithelium of the kidneys. Dosing increased the incidence of parathyroid hyperplasia in dosed males. The incidence of fibrous osteodystrophy of the bone also increased. This pathology was probably secondary to the renal toxicity and the resulting hyperparathyroidism. The incidences of carcinoma or squamous cell carcinoma of the Zymbal gland was increased in males. Alveolar epithelial hyperplasia occurred at a greater incidence accompanied by an increase in alveolar or bronchiolar adenomas in males. Dosed female rats had squamous cell papillomas of the oral cavity. Dosing caused diffuse hypertrophy of the thyroid gland in male rats. Follicular cell adenomas or carcinomas (combined) were increased. An increased number of cysts in the preputial gland were observed in high dose male rats. Dosing caused chronic inflammation, ulcers, and epithelial hyperplasia in the stomachs of males. Lastly, interstitial cell tumors were increased in the testes of dosed males. The doses in this study were many times higher than the proposed dose in the current NDA.

In a prospective study of 1373 patients given oral 8-methoxypsoralen photochemotherapy for psoriasis, thirty patients developed a total of 48 basal cell or squamous cell carcinomas. The observed incidence of cutaneous carcinoma was 2.63 times that expected for an age, sex and geographically matched population. Patients with a previous cutaneous carcinoma had a relative risk of 10.22.

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Recommendation:

The pharmacological or toxicological information provided in this submission is sufficient to establish that this therapy can be approved for palliative treatment of the skin manifestations of Cutaneous T-Cell Lymphoma.

Labeling Comments:

I will describe needed changes in the label for this therapy in a separate review.

/S/

W. David McGuinn, Jr., Ph. D., D.A.B.T.
February 19, 1999
Modified February 24, 1999

cc: IND 40,482
NDA 20-969
HFD-150 Division File
/W D McGuinn
/P Andrews
/S Hirschfeld
/D Catterson

February 24, 1999

/S/

2/24/99

Histopathology check list for UVADEX					
NDA 20-969					
			Submission date February 25, 1998		
Study	Langner et al	Thirteen Week Study	4-Week Study	Rozman et al.	Carcinogenicity
Species	Mouse	Rat	Dog	Monkey	Rat
Adrenals		X	X	X	X
Aorta			X	X	X
Axillary lymph nodes			X		
Bladder					
Bone				X	
Bone Marrow smear		X	X	X	X
Brain		X	X	X	X
Brain Stem			X		
Bronchi		X			X
Cecum			X		X
Cerebellum					
Cerebrum					
Cervix			X		
Colon		X		X	X
Clitoral or Preputial gland					X
Costchondral junction					X
Duodenum			X	X	X
Epididymis			X		X
Esophagus		X	X	X	X
Eye		X	X	X	X
Falopian tube					
Femur					
Gall bladder			X	X	
Gross lesions		X	X		X
Heart		X	X	X	X
Harderian gland					
Ileum			X	X	X
Injection site			X		
Jejunum			X	X	X
Kidneys	X	X	X	X	X
Lachrymal gland			X		
Larynx					X
Lymph node					
Liver	X	X	X	X	X
Lungs		X	X	X	X
Masses		X			X
Mammary Gland		X	X	X	X
Mandibular lymph node		X			X
Mesenteric lymph node		X	X	X	X
Nasal cavity					X
Optic nerve				X	
Oral cavity					X

Sheet1

Ovaries		X	X	X	X	
Pancreas		X	X	X	X	
Parathyroid		X	X		X	
Peripheral nerve				X		
Pharynx					X	
Pituitary		X	X	X	X	
Prostate		X	X	X	X	
Rectum				X	X	
Retropharyngeal lymph node			X			
Salivary gland		X	X	X	X	
Sciatic nerve			X		X	
Scrotal sack					X	
Seminal vesicle					X	
Skin	X			X		
Skeletal muscle				X		
Small Intestine		X				
Spinal cord		X	X	X	X	
Spleen		X	X	X	X	
Sternae		X			X	
Sternum			X			
Stomach	X	X	X	X		
Testes		X	X	X	X	
Thigh Muscle		X			X	
Thymus		X	X	X	X	
Thyroid		X	X		X	
Tongue		X	X	X	X	
Tracheobronchial lymph node			X			
Trachea		X	X	X	X	
Tunica vaginalis					X	
Unnary bladder		X	X	X	X	
Uterus		X	X	X	X	
Vagina			X			
Vertebrae					X	
Zymbal gland		X			X	
other						
other						