UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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CASWELL FILE

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SUBJECT: NCI Oncogenicity Study on Captan Caswell No 159

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The recent NIC report on the bioassay of Captan for possible carcinogenicity was presented for an evaluation. This bioassay was conducted by Gulf South Res. Inst., New Iberia, La. under contract to NCI. Osborne-Mendel rats and B6C3F1 mice were tested. The evaluation of this bioassay is presented below:

Protocol: 50 animals of each sex were used in test groups.

a). Rat Study:
Rats were fed diet containing Captan as outlined in the following table:

Table 1 a

Group	rats/sex	Captan (PPM)	Weeks treated	Weeks untreated	Time Weight Av. (PPM)
matched control	10	0	____	114	
		4,000	21		
Low dose	50	2,000	59		2,525
		0		33	
high dose	50	8,000	41		
		4,000	39		6050
		0		34	

Doses were lowered at the time indicated, based on projected probable mortality.

b). Mice Study:
Mice were fed diet as outlined below:

Table 1 b

Group	mice/sex	Captan (PPM)	Weeks treated	Weeks untreated
matched control	10	0	____	90-91

TABLE Cont. pg. 1

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Group	mice/sex	Captan (PPM)	Weeks treated	Weeks untreated
Low dose	50	8000	80	
		0		11
High dose	50	16.000	80	
		0		11

Rats were caged individually and mice were gang housed. Animals were fed an acetone treated diet containing 2% corn oil. The high dose in each case was the MTD derived from a 6 weeks feeding study with 5 animals/sex/group. The MTD for both rats and mice was initially estimated as 16,000 PPM of Captan. Accordingly both rats and mice were fed a diet of 8,000 and 16,000 PPM Captan. These dose levels were found to be too toxic to rats and consequently, the high dose groups were discontinued after 18 weeks. The groups of rats on 8,000 PPM Captan were then re-assigned as the high dose groups, (see table 1). Due to mortality, 7 males and 1 female in this dose level were replaced by healthy animals from the discarded 16,000 PPM groups.

The no. of animals in the matched controls were exceedingly small. Pooled control animals from studies on other chemicals conducted in the Laboratory were used for comparisons.

All animals were observed and examined as outlined below:

Signs of toxicity: twice daily
Body weight and palpation: at regular intervals
necropsy and histopathology: all moribund animals and those killed at termination. The following tissues and any lesion found were subjected to microscopic examination:

Skin	Liver	Adrenal
Lungs	Gall bladder	Thyroid
Bronchi	Pancreas	Parathyroid
Trachea	Stomach	Mammary gland
Bone and Marrow	Small intestine	Prostrate or uterus
Spleen	Large intestine	Testis or Ovary
Lymph nodes	Kidney	Brain
Heart	Urinary bladder	
Salivary gland	Pituitary	

Data obtained were subjected to the appropriate statistical analysis.

Results:

a). The body weight gain for M & F rats for both treatment groups were lowered. Certain clinical signs, eg rough hair coats, alopecia, pale mucous membranes, dermatitis, tachypnea and hematuria

were seen after the 1st year. A few female rats showed vaginal bleeding. There was no significant difference between control and treated rats in survival over 114 weeks.

Histopathologic findings showed no statistical difference between control and treated rats. There were a number of non-neoplastic lesions observed but thest were not related to treatment. For a summary of the tumor incidence for the rat study, see tabels II-V presented below:

Tabel II; Tumor Incidence in Male Rats.

	Low dose control	High dose control	Low	High
Number killed before termination.	1	2	21	27
Number at termination	4	3	29	23
Rats with 1^0 tumors	2	2	28	20
Number of 1^o tumors	2	3	32	24
Rats with 2^0 tumors	—	—	1	1

Table III, Incidence of the more frequent Primary Tumors in Male Rats.

tissue:	histopath	poded control	low dose	high dose	remark
thyroid	C-cell adenoma	2/65 (3)	1/42(2)	1/47 (2)	N.S.
pancreas	islet-cell adenoma	3/72 (4)	1/45(2)	1/47 (2)	N.S.
pituitary	chromophobe adenoma	8/62 (13)	9/43 (21)	5/45 (11)	N.S.
Liver	neoplastic nodule	2/73 (3)	1/47 (2)	2/49 (4)	N.S.

Table IV, Tumor Incidence in Female Rats

	low dose control	high dose control	low dose	high dose
No. killed before term	2	0	12	12
No. at termination	3	5	38	38
rats with 1^{o} tumors	3	4	36	32
No. 1^{0} tumors	4	4	47	41
rats with 2^{o} tumors	—	—	1	1
No. 2^{0} tumors	—	—	6	1

Table V, Incidence of Specific Primary Tumors (that were at least 5% in any one group) in female Rats.

tissue:	histopath	pooled control	low dose	high dose	remarks
pituitary	Chromophobe adenoma	12/62 (19)	12/48 (25)	4/45 (9)	—
liver	neoplastic nodule	1/71 (1)	4/49 (8)	0/50 (0)	—
adrenal	cortical adenoma or carcinoma	0/64 (0)	2/50 (4)	3/47 (6)	trend
pancreas	islet-cell adenoma	1/69 (1)	0/45 (0)	3/48 (6)	—
Mammary	adenoma or adenocarcinoma I.D. carcinoma,Nos.	0/67 (0)	3/50 (6)	3/50 (6)	—
mammary	fibroma	1/72 (1)	2/50 (4)	3/50 (6)	—
mammary	fibroadenoma	8/72 (11)	4/50 (8)	5/50 (10)	—
mammary	adenoma, nos, or fibroadenoma	8/72 (11)	7/50 (14)	6/50 (12)	—
uterus	endometrial stromal polyp	7/67 (10)	6/48 (13)	7/45 (16)	—
thyroid	C-cell adenoma	1/66 (2)	1/49 (2)	4/44 (9)	trend

Tissue:	Histopath	pooled control	low dose	high dose	remarks
thyroid	C-cell adenoma or carcinoma	2/66 (3)	2/49 (4)	4/44 (9)	

Evaluation: It may be concluded that there is no evidence of tumor induction by Captan in male or female rats. Certain trends are seen in tumor formation related to treatment, eg the adrenal cortical tumors and the thyroid c-cell adenoma in female rats.

This study may be deemed as acceptable but it suffers from several major short comings. These are:

(i) the 18 months treatment period for rats is relatively short.

(ii) the very small no. of animals in the matched control groups

(iii) the replacement of animals for the 8,000 PPM groups by healthy animals from the discontinued 16,000 PPM groups.

(iv) the MDT was poorly determined.

(v) the low dose animals were put on test 20 weeks after the high dose animals.

Results:

b). Mice Study: The body weight gain for male and female mice on the high dose was clearly lowered. A small effect on growth was seen for the low dose animals. Various behavoral and clinical signs became observable as the study progressed. There was no dose related effect on mortality. More than 90% of the animals remained alive at the end of the study. Tumor incidences were summarised in Tables VI-IX.

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Table VI ; Tumor Incidence in male Mice.

	control	low dose	high dose
no. killed before term	1	3	2
no. at termination	9	47	48
no: mice with 1^o tumors	5	7	10
no. 1^o tumors	5	7	10
no. mice with 2^o tumors	1	--	--
no. 2^o tumors	1	--	--

Table VII ; Incidence of Specific Primary Tumors (that was > 5% in any one group) in male Mice.

tissue: histopath	pooled control	low dose	high dose	remark
lung alv/bronc adenoma or carcinoma	5/66 (8)	3/47 (6)	1/49 (2)	
liver neopl. nodules heptato-carcinoma	14/76 (18)	1/46 (2)	3/49 (6)	
duodenum I adenomatous polyp, nos.	0/68 (0)	2/43 (5)	2/46 (4)	
duodenum II adenocarcinoma in adenomatous polyp	0/68 (0)	1/43 (2)	3/46 (7)	
duodenum I & II	0/68 (0)	3/43 (7)	5/46 (11)	$P < 0.01$

Table VIII ; Tumor Incidence in female Mice.

	control	low dose	high dose
no. killed before term	1	2	4
no. at termination	9	48	46
mice with 1^o tumors	3	5	8
no. 1^o tumors	3	5	8

Table IX ; Incidence of Primary Tumors (that was > 5% in any one group) in Female Mice.

tissue: histopath	pooled control	low dose	high dose	remark
liver neoplastic nodules	2/67 (3)	1/49 (2)	1/47 (2)	
duodenum I adenomatous polyp, nos	1/68 (1)	1/49 (2)	0/48 (0)	

tissue: histopath	pooled control	low dose	high dose	remark
duodenum II adenocarcinoma in adenomatous polyp	0/68 (0)	0/68 (0)	3/48 (6)	
duodenum I & II	1/68 (1)	1/49 (2)	3/48 (6)	

Notes: Nos. ___ not otherwise specified.

Evaluation: The incidence of duodenal tumors in male mice is significantly increased as a result of Captan ingestion. In female mice, this increase is not statistically significant. In contrast to the rat bioassay, this study is relatively well performed despite the fact that the size of the matched control groups is exceedingly small. The low incidence of spontanous duodenal tumors in B6F3C1 mice;

male = 0.23% (of 2334 mice)
female = 0.30% (of 1985 mice)

also lends support to the conclusion that Captan is a weak oncogen for male mice. The oncogenic potential is seen to be statistically significant at 16,000 PPM and not at 8,000 PPM of the fungicide.

Conclusion: The increase in the incidence of duodenal tumors in the male B6F3C1 mice is treatment related, significantly at a dose of 16,000 PPM but not at 8,000 PPM of Captan. For rats and also for female mice no such an effect was produced at the MTD levels. Similarly no positive oncogenic potential was shown by the study completed by Innes et al (1969). It should be pointed out that in this study, possible duodenal lesions may not have been examined as carefully. In addition, both the dose levels and the number of mice per dose level were substantially smaller. Consequently, the oncogenic potential may not have been fully assessed. Two oncogenicity studies, for rats and mice respectively, have been performed by IBT (Reyna et al, 1974 a, b). Among other factors, the dose levels tested were lower than the most recent NCI studies. From these studies, an adequate evaluation of the oncogenic potential of Captan is not possible.

The current results from NCI should be regarded as the best available evidence that places Captan as a weak carcinogen.

However, the oncogenic potential of Captan should not be viewed in isolation. There is evidence that Captan is rapidly detoxified in Vivo (Legator and Zimmering 1975, Bridges, 1975; Ficsor et al, 1977)

This is reflected in its low order of toxicity seen in general, in acute to chronic studies via the oral route of intake. Additional support for Captan deactivation comes from certain mutagenic studies. Captan has been shown to be a direct acting mutagen in several Vitro

assay systems (Bridges, 1975; Fahrig, 1974; Ficsor et al, 1977 and Legator and Zimmering, 1975). This action is however abolished in the presence of a liver microsomal preparation intended for metabolic activation or after incubation with human or rat blood (Ficsor et al, 1977; Luken and Sisler 1958 and DeBaun et al, 1974). Ficsor et al (1977) could not detect any mutagenic activity in blood samples taken 10-180 mins. after hefty doses of Captan, ie 1000 mg/kg I.P. or 2000 mg/kg P.O. Indeed the t 1/2 of deactivation for Captan in blood for mutagenic activity has been estimated to be < 1 min. There are good indications that the thio - SH group in the biological system is mainly responsible for the deactivation. Other groups in the tissue may also play a role. Antagonism to other inhibitory actions of Captan in Vitro has been shown by the addition of thiol group (Gale et al 1971). The reaction of Captan with the thiol group is believed to proceed as follow:

The toxophore in the molecule is thus postulated to be the trichloromethyl thio (-S-C Cl3) group (BeDaun et al 1974). In view of the sharply contrasting potencies of Captan seen in Vitro mutagenic and in Vivo oncogenic studies (Bridges, 1975 and NCI report No. 15, 1977) it is not unlikely that the oncogenic activity of the fungicide may be detoxified in vivo. A more definitive conclusion can not be reached in the absence of actual studies.

Many conflicting results on the toxic effects of Captan in in Vivo studies have been obtained. The more important ones are cited below:

(i) Both positive and negative results have been obtained for host mediated mutagenic assays (Bridges, 1975 Fahrig, 1974). Buslemaier et al (1972), employing Salmonella G-46 his. strain were able to show a positive mutagenic effect with an I.M. application of a near lethal dose of 500 mg/kg to rats. Other attempts to obtain such a result were unsuccessful, (Ficsor et al, 1977).

Similar observations have been made for the dominant lethal studies in rats and mice, (Collins, 1972 a) and Epstein and Shafner 1968). Collins, (1972 a) obtained a dominant lethal mutation in mice and possibly in rats as well at doses of 100 mg/kg P.O. and 10 mg/kg I.P. No detectable effect was produced at lower doses.

Positive heritable translocation mutation in mice has been shown at 5000 PPM of Captan (or ≈ 700 mg/kg) but not at 2500 PPM (≈ 350 mg/kg) (Jorgensen et al, 1977). The parameters observed include a significant increase in the no. of dead implants and in the no. of presumptive

sterile males seen in the F_1 breeding results. Another polygenic mutation study, also in mice, shows a possible translocation effect, seen in the lowering of weaning weight at 50 mg/kg and in the decrease in survival index as well at 100 mg/kg (Collins 1972 b) for the F_2 generation. In addition, a dominant lethal effect is observed at 50 mg/kg in the F_1 generation. The large discrepancy in the effect dose levels between these 2 studies needs to be resolved. The effect dose levels from Collins (1972 b) ie 50 mg/kg if reproducable, should place the mutagenic potential of Captan as a more imminent hazard than any other toxic effect of the fungicide.

(ii), Conflicting results on the teratogenic potential of Captan have been obtained in a no. of Mammaliau species. (McLaughlin Jr. et al, 1969; Fabro et al 1966, Courtney et al, 1970 and Robens, 1970). The lowest NEL in studies where a teratogenic potential has been shown is in the region of 37.5 mg/kg for rabbits (McLaughlin Jr. et al, 1969). Teratogenicity studies on a half dozen mammalian species have been reported by IBT. These studies can not be evaluated for obvious reasons.

It may be possible that one of the major factors contributing to these confusing phenomena is the presence of the detoxification system. No investigations however, have been directed to relate any of the conflicting toxic effects to the detoxification potential of the various strains and species of animals used. Of equal significance is the complete lack of any evidence that intact Captan alone is responsible for the mutagenic, oncogenic and teratogenic effects observed. The acqusition of information on these lines may prove to be difficult but attempts should be initiated.

The oral route of exposure to Captan has been more extensively studied. A low potential has been demonstrated for many aspects of toxicity, including oncogenicity. However, certain results obtained from some mutagenic and teratogenic studies have rendered the evalutation of the safe use of the fungicide, incomplete (Collins, 1972 a,b McLaughlin Jr. et al 1969 and Robens, 1970). In these studies refered to, NEL (s) have not been shown for the mutagenicity testing and probably also not for teratology in rabbits had single dosing been used. Consequently, the lack of possible hazard that may arise from the tolerances established in/on the various crops and food items (see attached computer printout) cannot be on firm ground. In view of the availability of additional studies that have not been evaluated by E.P.A., the ADI should be reprocessed. Additionally, the actual current dietary exposure to Captan and its major metabolites should be determined if possible. A previous monitor placed the level of Captan as 0.178 PPM (Pesticides Monit. J. 10. 137 (1977). This effort however, failed to measure for Captan metabolites, which, inview of the short t 1/2 of the fungicide should be a more appropriate reflection of Captan residues.

The long term effects of Captan through the dermal and inhalational routes are unknown. Similarly, the possible presence and capacity of a detoxification system along these sites have not been approached. In as much as these 2 routes of intake are related to occupational personnel, in the absence of adequate information, the exposure must be mimmised

Through the sue of coveralls and respirators: Further more, the extent of exposure in occupational groups should be determined.

We note that Captan is at the moment undergoing the RPAR process In EPA. It is reasonably well established that Captan is a weak oncogen. However, the areas that should receive more attention in relation to possible hazards are the mutagenic potential as seen in certain dominant lethal and polygenic translocation studies (Collins, 1972, a, b,) and possibly the teratogenic activity (McLaughlin Jr. et al 1969). The possibility of the existence of a detoxification system in Vivo for the fungicide should deserve considerations.

Recommendations: These should be contigent on OSPR concurrence.

(1). Comprehensive studies, encompassing the parameters examined by Collins (1972 a, b) and McLaughlin Jr. et al (1969) should be conducted. For teratogenicity testing, both single and multiple dosings should be used. There studies, in more than 1 species, should be coupled to the corresponding metabolism/detoxification studies with particular attention on possible tissue blinding, (especially in the gonads) placenta crossing and binding/retention in the embryo of any metabolic derivative of the tetrahydorphthalimide and the trichloromethylthio groups.

(2). Similarly, metabolism studies should be conducted on repeated dermal and inhalational applications with emphasis on excretion, excretory products and tissue binding of any metabolic derivative of the terrahydrophthalimide and the trichloromethylthio groups.

(3). The ADI should be re-evaluated in light of the availability of several studies (see 1) that have not been considered by EPA previously.

(4). The actual dietary intake of Captan residues from tolerances established should be ascertained. The extent of exposure by occupational groups should be determined.

(5). The exposure to Captan by occupational groups should be mimmised by the use of protective coveralls and respirators, as soon as feasible.

(6). The 2 closely related chemicals, folpet and difolatan should be evaluated by EPA.

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