

Results of Long-Term Experimental Studies on the Carcinogenicity of Ethylene-bis-Dithiocarbamate (Mancozeb) in Rats

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ABSTRACT: Mancozeb, an ethylene-bis-dithiocarbamate (EBDC), has been one of the most commonly used fungicides in commercial use for several decades. Nevertheless, up to now, no adequate published experimental studies on the carcinogenicity of Mancozeb have been published. Because of the importance of the compound and of the number of people potentially exposed (workers engaged in the production and use of the fungicide, people living in agricultural areas where the compound is sprayed, and people consuming polluted products), a long-term experimental study of Mancozeb was begun at the Cancer Research Center of the Ramazzini Foundation. Groups of 150 male and female Sprague-Dawley rats, 8 weeks old at the start of the treatment, were administered Mancozeb at the concentration of 1000, 500, 100, 10, and 0 ppm in feed supplied *ad libitum* for 104 weeks. At the end of the treatment, animals were kept under controlled conditions until spontaneous death. Mancozeb caused an increase in (1) total malignant tumors, (2) malignant mammary tumors, (3) Zymbal gland and ear duct carcinomas, (4) hepatocarcinomas, (5) malignant tumors of the pancreas, (6) malignant tumors of the thyroid gland, (7) osteosarcomas of the bones of the head, and (8) hemolymphoreticular neoplasias. On the basis of these data, Mancozeb must be considered a multipotent carcinogenic agent.

KEYWORDS: ethylene-bis-dithiocarbamate; Mancozeb; carcinogenicity; long-term bioassay; rat

INTRODUCTION

The issue of long-term toxicity and carcinogenicity of pesticides has been one of the major problems of public health in the last two decades. Unfortunately, only a small percentage of these compounds are tested for toxicity or carcinogenicity prior to their production, distribution, and general use. There is extensive scientific documentation to show that pesticides, especially during production, application, and the

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disposal of containers, may be released into the general environment and be present at measurable concentrations in the atmosphere, soil, surface and ground waters, and as residues in food.

Ethylene-bis-dithiocarbamate (Mancozeb) is one of the most widely used commercial fungicides worldwide and is used in agriculture in Italy, particularly in the Emilia Romagna Region. In spite of its widespread use, few data exist on the carcinogenicity of this compound. This experiment was designed to evaluate the carcinogenic effects of Mancozeb because of the importance of the compound, the consumption in our country and region, and the number of people exposed that include workers engaged in the production and use of the compound, citizens living in agricultural areas where the compound is sprayed, and citizens consuming polluted products.

Mancozeb ($C_4H_6MnN_2S_4$)_a(Zn)_y is a fungicide that belongs to the ethylene(bis) dithiocarbamate family. It is a zinc ion coordination product with a manganese ethylene-1,2-bis-dithiocarbamate polymer and has a molecular weight of $265.3 + 65.4$. Mancozeb and similar substances were first marketed in 1944, and their usage increased steadily. After the introduction of systemic fungicides, use of bis-dithiocarbamates declined temporarily, but as resistance to systemic fungicides developed, the use of bis-dithiocarbamates, mainly Mancozeb, increased, and it is now one of the most widely used fungicide products.¹ Mancozeb acts by enzyme activity inhibition. It is synthesized^{2,3} from carbamate radicals that have reacted with carbon disulfide to give dialkyldithiocarbamates. The reaction with diamines gives dithiocarbamate groups. The addition of zinc chloride to a suspension of dithiocarbamate, named Maneb, yields a product named Mancozeb, which is superior to Maneb in fungicide activity. The world production of Mancozeb in 1983 was about 30,000 tons.⁴

Because of its efficacy against a broad spectrum of fungi and their associated plant diseases,^{2,3} Mancozeb is widely used as a fungicide in agriculture on a variety of horticultural crops and wheat. It is also used in industry as a slimeicide in water-cooling systems; in sugar, pulp, and paper manufacturing; as a vulcanization accelerator and antioxidant in the rubber industry; and as a scavenger in wastewater treatment because of its chelating properties.³

Mancozeb is not known to occur as a natural product. It has a negligible vapor pressure and a low potential to volatilize into the environment.^{5,6} It can be found associated with air-borne particulates or as spray drift.⁵ Mancozeb has low solubility in water but hydrolyzes rapidly over a wide range of pH.⁵ It is hydrolyzed within 1 day and has a field half-life of 1 to 7 days.⁵ It has many metabolites, of which the most important is ethylenethiourea (ETU),⁷ which has a high water solubility.⁵ Mancozeb has low soil persistence due to its fast hydrolysis and has moderate adsorption capacity, whereas ETU is potentially mobile through the soil and can result in groundwater pollution.⁵ Residues are regularly detected in fruit and vegetables, and it has been shown that a significant percentage of ETU is produced during cooking of contaminated vegetables.⁸

Oral administration to rats showed an $LD_{50} > 800$ mg/kg of body weight.⁹ Administered by inhalation or ingestion at different dose levels for 13 weeks to rats and mice, Mancozeb altered thyroid hormone levels and produced diffuse thyroid follicular epithelial hyperplasia.¹

No adequate study exists to evaluate reproductive effects over at least two generations.¹⁰ In a teratogenicity study, pregnant Sprague-Dawley rats, treated by gavage

at the 11th day of pregnancy with a single administration of Mancozeb at doses of 0–1320 mg/kg b.w., showed an incidence of offspring with gross malformations of 25% in the group treated with the highest dose.¹¹ In some studies, Mancozeb has been found to cause chromosome aberrations and sister chromatid exchanges in workers occupationally exposed.¹²

Although Mancozeb has been commercially produced for almost 60 years, the carcinogenicity studies on this fungicide are, in our opinion, still inadequate. To date, Mancozeb carcinogenicity experiments have been conducted on rats and mice.

Charles River Crl:CDBR rats received 0–750 ppm of a technical product of Mancozeb (83.8% pure) for two years. Thyroid follicular cell adenomas and/or carcinomas were noted in both sexes at the highest dose level.¹³ Sprague-Dawley (CD) rats were given 0–400 ppm of the same mixture. No evidence of tumorigenicity was observed.¹³ In two experiments, groups of Charles River CD-1 mice were given 0–1000 ppm dietary concentrations for 78 weeks. No different incidence of liver nodules and liver masses was observed between treated and control animals; overall, there was no evidence of carcinogenicity.¹³

Studies on the carcinogenicity of ETU, the Mancozeb contaminant, degradation product, and metabolite, were conducted on rats and mice. Charles River rats were treated for 78 weeks with feed containing ETU at concentrations of 0–350 ppm and kept alive for an additional 26 weeks on a controlled diet. At the higher dose, the incidence of thyroid carcinomas was 17% in males and 8% in females. No thyroid tumor was observed in control animals in either sex.¹

Charles River rats were treated for 104 weeks with feed containing ETU at concentrations of 0–500 ppm. The incidence of thyroid carcinoma was 62% in rats treated with 500 ppm, 16% in those treated with 250 ppm, and between 1% and 2% in animals treated with the lowest doses and in the control group.¹

Combined perinatal and two-year adult dietary exposure to ETU caused Fischer 344/N rats of each sex to have an increased incidence of thyroid neoplasms and a marginal increase in Zymbal gland neoplasms and mononuclear cell leukemias. In a two-year adult-only dietary exposure of Fischer rats 344/N to ETU, an increased incidence of thyroid follicular cell neoplasms occurred in males and females.¹⁴

Combined perinatal and two-year adult dietary exposure to ETU caused male and female B6C3F₁ mice to have an increased incidence in thyroid follicular cell neoplasms, hepatocellular neoplasms, and adenomas of the pars distalis of the pituitary gland.¹⁴

It must be noted that all the experiments on Mancozeb technical products and ETU were truncated at 104 weeks. The possibility of tumor development in later life was not evaluated.

MATERIALS AND METHODS

Mancozeb was supplied by the Rohm and Haas Company, Philadelphia, PA, USA. Its purity was 85%, as active ingredient. The general protocol of the experiment has been described in detail in this volume.¹⁵ Mancozeb was added at concentrations of 1000, 500, 100, 10, or 0 ppm to the standard “Corticella” diet, used for 30 years at the laboratories of the Cancer Research Center of the Ramazzini Foundation (CRC/RF), and prepared and supplied monthly by the “Laboratorio Dottori

Piccioni." Mancozeb-treated feed was administered *ad libitum* to Sprague-Dawley rats (75/sex/group), 8 weeks old at the start of the experiments. Control animals received the same feed without Mancozeb. Treated animals were given Mancozeb-treated food for 104 weeks after which time they received standard food. All animals were kept under observation until spontaneous death.

RESULTS

There were no differences between the various groups in mean daily water or food consumption, body weight, survival, or behavior. No treatment-related nononcological pathological changes were detected by gross inspection during the experiment or by histopathological examination.

The occurrence of benign and malignant tumors among male and female rats in treated and control groups is shown in TABLE 1. Differences observed among treated animals and controls were:

(1) increases in total malignant tumors in males and in females in all of the Mancozeb-treated groups—in males, the increase was dose-related (TABLE 2);

(2) increases in total malignant mammary tumors in all Mancozeb-treated females—the differences were statistically significant at the dose levels of 1,000 and 100 ppm;

(3) increased incidence in Zymbal gland and ear duct carcinomas in males treated at 1000 ppm; an increased incidence of carcinomas was also observed in nasal and oral cavities, tongue, lips, pharynx, and larynx. Overall, the incidence of head and neck carcinomas increased in males (dose-related) and in females of all treated groups (TABLE 3);

(4) increased incidence of hepatocarcinomas in males at the highest dose (1000 ppm);

(5) increased incidence of malignant tumors of the pancreas in males and females treated with 1000, 500, and 100 ppm—one case of islet cell carcinoma was observed in a female treated with 10 ppm;

(6) increased incidence of malignant tumors of the thyroid gland in males treated with 1000 and 500 ppm, and in females treated with 1000, 100, and 10 ppm (TABLE 4);

(7) increased incidence of osteosarcomas in bones of the head in males treated with 1000, 500, 100, and 10 ppm and in females treated with 1000, 500, and 10 ppm—the differences were statistically significant in males and females treated at 10 ppm;

(8) increases in hemolymphoreticular neoplasias (lymphomas and leukemias) in males and females treated with 1000, 500, 100, and 10 ppm of Mancozeb (TABLE 5).

CONCLUSIONS

Animals treated with Mancozeb in food from age 8 weeks through age 104 weeks and followed until spontaneous death showed a significant increase in total tumors and in tumors of specific type that were often sex specific. Mancozeb was shown to be carcinogenic on the basis of the number of total malignant tumors and the tumors

TABLE 1. Long-term carcinogenicity bioassay on Mancozeb administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats

		NUMBER AND PERCENTAGE OF MALE AND FEMALE SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS ^a																			
Site	Histotype	Groups																			
		I: 1,000 ppm				II: 500 ppm				III: 100 ppm				IV: 10 ppm				V: 0 (control)			
		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Skin																					
	Papilloma	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
	Trichoepithelioma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-
	Squamous cell carcinoma	1	1.3	0	-	0	-	0	-	2	2.7	0	-	0	-	0	-	0	-	0	-
	Dermatofibrosarcoma	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Subcutaneous tissue																					
	Fibroma	1	1.3	0	-	1	1.3	1	1.3	0	-	1	1.3	1	1.3	0	-	0	-	0	-
	Lipoma	2	2.7	0	-	0	-	0	-	1	1.3	0	-	0	-	0	-	1	1.3	1	1.3
	Fibrosarcoma	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-
	Liposarcoma	1	1.3	0	-	1	1.3	0	-	1	1.3	0	-	1	1.3	0	-	4	5.3	0	-
Mammary glands																					
	Fibroma & fibroadenoma	3	4.0	43(72)	57.3	3	4.0	32(51)	42.7	5	6.7	30(44)	40.0	4	5.3	41(64)	54.7	2	2.7	35(54)	46.7
	Lipoma	2(3)	2.7	0	-	3	4.0	0	-	2	2.7	1	1.3	2	2.7	0	-	3(4)	4.0	0	-
	Adenocarcinoma	1	1.3	5(7)	6.7	0	-	8(10)	10.7	0	-	9(13)	12.0	0	-	5	6.7	1	1.3	3	4.0
	Fibrosarcoma	1	1.3	2	2.7	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-
	Liposarcoma	1	1.3	2	2.7	1	1.3	0	-	1	1.3	0	-	1	1.3	0	-	2	2.7	0	-
Zymbal glands^b																					
	Sebaceous adenoma	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-
	Carcinoma	12	16.0	5(6)	6.7	6	8.0	6	8.0	4(5)	5.3	4	5.3	1	1.3	6	8.0	1	1.3	1(2)	1.3
Ear ducts^b																					
	Acanthoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-
	Carcinoma	10	13.3	11	14.7	7	9.3	11(12)	14.7	5(6)	6.7	7(8)	9.3	8(10)	10.7	10	13.3	2	2.7	6	8.0
Nasal cavities^b																					
	Carcinoma	1	1.3	0	-	1	1.3	1	1.3	1	1.3	0	-	1	1.3	0	-	0	-	0	-
	Olfactory neuroblastoma	0	-	0	-	1	1.3	1	1.3	0	-	0	-	1	1.3	0	-	0	-	0	-
Oral cavity, tongue & lips																					
	Carcinoma	2	2.7	4	5.3	3	4.0	0	-	1	1.3	1	1.3	0	-	3	4.0	2	2.7	0	-
	Fibrosarcoma	0	-	0	-	0	-	5	6.7	0	-	3	4.0	0	-	0	-	1	1.3	1	1.3
Pharynx^b																					
	Carcinoma	0	-	1	1.3	0	-	0	-	0	-	1	1.3	1	1.3	1	1.3	0	-	0	-
Larynx^b																					
	Carcinoma	0	-	0	-	1	1.3	0	-	1	1.3	0	-	0	-	4	5.3	0	-	0	-
Lung																					
	Adenoma	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-
	Adenocarcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	1	1.3
	Fibrosarcoma	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-

— Continued

TABLE 1. Continued

Site	Groups																			
	I: 1,000 ppm		II: 500 ppm		III: 100 ppm		IV: 10 ppm		V: 0 (control)											
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female										
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%									
Stomach																				
- Forestomach																				
Acanthoma	2	2.7	4	5.3	2	2.7	3	4.0	3	4.0	2	2.7	4	5.3	3	4.0	5	6.7	8	10.7
Adenoma	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-
Squamous cell carcinoma	1	1.3	0	-	1	1.3	0	-	2	2.7	0	-	0	-	0	-	1	1.3	0	-
Inesime																				
Adenomatous polyp	0	-	1	1.3	0	-	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-
Adenocarcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	1	1.3
Leiomyosarcoma	0	-	1	1.3	0	-	0	-	1	1.3	0	-	0	-	0	-	1	1.3	0	-
Salivary glands																				
Adenocarcinoma	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Liver																				
Cholangioma	1	1.3	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Hepatocarcinoma	4	5.3	0	-	1	1.3	0	-	1	1.3	2	2.7	0	-	0	-	0	-	0	-
Angiosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3
Pancreas																				
Exocrine adenoma	0	-	0	-	4	5.3	2	2.7	0	-	0	-	0	-	0	-	0	-	3	4.0
Islet cell adenoma	10	13.3	5	6.7	10	13.3	7	9.3	10	13.3	6	8.0	8	10.7	8	10.7	5	6.7	11	14.7
Exocrine adenocarcinoma	1	1.3	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Islet cell carcinoma	3	4.0	0	-	2	2.7	2	2.7	1	1.3	3	4.0	0	-	0	-	1	1.3	0	-
Kidneys																				
Adenoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3
Nephroblastoma	0	-	0	-	0	-	1	1.3	1	1.3	0	-	0	-	0	-	0	-	0	-
Adenocarcinoma	0	-	0	-	1	1.3	1	1.3	0	-	1(2)	1.3	0	-	0	-	0	-	0	-
Liposarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-
Bladder																				
Papilloma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Leiomyosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-
Prostate																				
Adenocarcinoma	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-
Testes																				
Interstitial cell adenoma	7(8)	9.3	10	13.3	10	13.3	6(8)	8.0	6(7)	8.0	8.0	8.0	6(7)	8.0	7(8)	9.3	9.3	9.3	9.3	9.3
Ovaries																				
Cystadenoma	3	4.0	0	-	0	-	3	4.0	0	-	0	-	0	-	0	-	0	-	0	-
Granulosa cell tumor	1	1.3	0	-	1	1.3	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Sertoli cell tumor	2	2.7	0	-	4(5)	5.3	5	6.7	5	6.7	1	1.3	1	1.3	1	1.3	6	8.0	6	8.0
Granulosa cell malignant tumor	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Malignant thecoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1.3	0	-	0	-

— Continued

TABLE 1. Continued

Site	Groups																				
	I: 1,000 ppm				II: 500 ppm				III: 100 ppm				IV: 10 ppm				V: 0 (control)				
	Male	No.	%	Female	Male	No.	%	Female	Male	No.	%	Female	Male	No.	%	Female	Male	No.	%	Female	
Peripheral nervous system	0	0	-	0	0	-	0	0	0	-	0	0	0	0	-	0	0	0	0	0	0
- Ganglia																					
Pheochromocytoma																					
Bones																					
- Head																					
Chondroma	0	0	-	0	0	-	0	2	2.7	0	-	0	0	0	-	0	0	0	0	0	0
Osteoma	0	0	-	0	1	1.3	0	0	0	0	-	0	0	0	-	0	0	0	0	0	0
Chondrosarcoma	0	0	-	0	0	-	0	0	0	0	-	0	1(2)	1.3	0	0	0	0	0	0	0
Osteosarcoma	8	10.7	3	4.0	5	6.7	4	5.3	8	10.7	1	1.3	13	17.3	8	10.7	2	2.7	1	1.3	1.3
- Other																					
Osteosarcoma	1	1.3	1	1.3	1	1.3	0	0	0	0	-	0	0	0	-	1	1.3	2	2.7	0	0
Soft tissues																					
Liposarcoma	1	1.3	0	0	1	1.3	0	0	0	0	-	0	0	0	-	0	1	1.3	0	0	0
Angiosarcoma	1	1.3	0	0	0	0	0	0	0	0	-	0	0	0	-	0	0	0	0	0	0
Heart																					
Myxosarcoma	1	1.3	0	0	0	0	0	0	0	0	-	0	0	0	-	0	0	0	0	0	0
Malignant Schwannoma	1	1.3	0	0	0	0	0	0	0	0	-	0	0	0	-	0	0	0	0	1	1.3
Thymus																					
Malignant thymoma ^a	0	0	-	0	0	-	0	0	0	0	-	1	1.3	0	0	-	2	2.7	0	0	0
Spleen																					
Fibrosarcoma	1	1.3	0	0	1	1.3	0	0	0	0	-	0	0	0	-	0	0	0	0	0	0
Angiosarcoma	1	1.3	0	0	0	0	1	1.3	1	1.3	0	0	0	0	-	0	0	0	0	0	0
Mesenteric lymph nodes																					
Fibrosarcoma	0	0	-	0	1	1.3	0	0	0	0	-	1	1.3	0	0	-	0	0	0	1	1.3
Hemolymphoreticular tissues ^{b, c}	30	40.0	16	21.3	35(37)	46.7	21	28.0	32	42.7	27	36.0	22	29.3	20	26.7	16	21.3	11	14.7	14.7
Lymphomas & leukemias																					

^a Numbers between brackets indicate total number of tumors; one animal can bear more than one tumour
^b See Table 3
^c See Table 4
^d One is bearing bilateral tumors
^e In 96% of cases the tumor itself is composed of a mixture in varying proportions of epithelial cells and lymphocytes. In the remaining 4%, only epithelial cells are present. We consider that a tumor composed exclusively of lymphocytes should not be classified as a thymoma but as a lymphoma involving the thymus
^f Including thymus, spleen and mesenteric lymph nodes
^g See table 5

TABLE 2. Long-term carcinogenicity bioassay on Mancozeb administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats

TOTAL MALIGNANT TUMORS							
Group No.	Concentration (ppm)	Animals		Malignant tumors			
		Sex	No.	Tumor-bearing animals		Tumors	
				No.	%	No.	Per 100 animals
I	1,000	M	75	59	78.7 ***♦♦	99	132.0 **
		F	75	43	57.3	84	112.0 **
		M+F	150	102	68.0	183	122.0
II	500	M	75	55	73.3 ***♦♦	78	104.0 **
		F	75	46	61.3 *	78	104.0 **
		M+F	150	101	67.3	156	104.0
III	100	M	75	51	68.0 ***♦♦	72	96.0 **
		F	75	42	56.0	74	98.7 **
		M+F	150	93	62.0	146	97.3
IV	10	M	75	39	52.0 ♦♦	57	76.0 *
		F	75	50	66.7 **	82	109.3 **
		M+F	150	89	59.3	139	92.7
V	0	M	75	29	38.7	38	50.7
		F	75	31	41.3	40	53.3
		M+F	150	60	40.0	78	52.0

* $p < 0.05$ using χ^2 test. ** $p < 0.01$ using χ^2 test. *** $p < 0.01$ using Cochrane-Armitage test for dose-response relationship.

TABLE 3. Long-term carcinogenicity bioassay on Mancozeb administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats

CARCINOMAS OF THE HEAD AND THE NECK																	
Group No.	Concentration (ppm)	Animals		Animals with carcinomas ^a													
		Sex	No.	Zybal glands		Ear ducts		Nasal cavities		Oral cavity, tongue and lips		Pharynx		Larynx		Total	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	1,000	M	75	12	16.0 **	10	13.3 *	1	1.3	2	2.7	0	-	0	-	25	33.3 ***♦♦
		F	75	5 (1)	6.7	11	14.7	0	-	4	5.3	1	1.3	0	-	21	28.0 **
		M+F	150	17	11.3	21	14.0	1	0.7	6	8.0	1	0.7	0	-	46	30.7
II	500	M	75	6	8.0	7	9.3	2 ^b	2.7	3	4.0	0	-	1	1.3	19	25.3 ***♦♦
		F	75	6	8.0	11 (1)	14.7	2 ^b	2.7	0	-	0	-	0	-	19	25.3 *
		M+F	150	12	8.0	18	12.0	4	2.7	3	2.0	0	-	1	0.7	38	25.3
III	100	M	75	4 (1)	5.3	5 (1)	6.7	1	1.3	1	1.3	0	-	1	1.3	12	16.0 ♦♦
		F	75	4	5.3	7 (1)	9.3	0	-	1	1.3	1	1.3	0	-	13	17.3
		M+F	150	8	5.3	12	8.0	1	0.7	2	1.3	1	0.7	1	0.7	25	16.7
IV	10	M	75	1	1.3	8 (2)	10.7	2 ^b	2.7	0	-	1	1.3	0	-	12	16.0 ♦♦
		F	75	6	8.0	10	13.3	0	-	3	4.0	1	1.3	4	5.3	24	32.0 **
		M+F	150	7	4.7	18	12.0	2	1.3	3	4.0	2	1.3	4	2.7	36	24.0
V	0	M	75	1	1.3	2	2.7	0	-	2	2.7	0	-	0	-	5	6.7
		F	75	1 (1)	1.3	6	8.0	0	-	0	-	0	-	0	-	7	9.3
		M+F	150	2	1.3	8	5.3	0	-	2	1.3	0	-	0	-	12	8.0

^aBetween brackets the number of animals with bilateral tumors. ^b1 olfactory neuroblastoma. * $p < 0.05$ using χ^2 test. ** $p < 0.01$ using χ^2 test. *** $p < 0.01$ using Cochran-Armitage test for dose-response relationship.

TABLE 4. Long-term carcinogenicity bioassay on Mancozeb administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats

ONCOLOGICAL LESIONS OF THE THYROID GLAND													
Group No.	Concentration (ppm)	Animals		Tumor-bearing animals									
		Sex	No.	Follicular adenomas		C-cell adenomas		Follicular carcinomas		C-cell carcinomas		Total	
				No.	%	No.	%	No.	%	No.	%	No.	%
I	1,000	M	75	10	13.3 ^{***}	4	5.3	6	8.0 [*]	2	2.7	22	29.3 ^{***}
		F	75	9	12.0 ^{**}	7	9.3	12	16.0 ^{***}	2	2.7	30	40.0 ^{***}
		M+F	150	19	12.7	11	7.3	18	12.0	4	2.7	52	34.7
II	500	M	75	3	4.0	4	5.3	2	2.7	0	-	9	12.0
		F	75	0	-	1	1.3	0	-	0	-	1	1.3
		M+F	150	3	2.0	5	3.3	2	1.3	0	-	10	6.7
III	100	M	75	1	1.3	3	4.0	0	-	1	1.3	5	6.7
		F	75	3	4.0	5	6.7	0	-	1	1.3	9	12.0
		M+F	150	4	2.7	8	5.3	0	-	2	1.3	14	9.3
IV	10	M	75	0	-	1	1.3	0	-	0	-	1	1.3
		F	75	2	2.7	5	6.7	1	1.3	0	-	8	10.7
		M+F	150	2	1.3	6	4.0	1	0.7	0	-	9	6.0
V	0	M	75	0	-	2	2.7	0	-	0	-	2	2.7
		F	75	1	1.3	4	5.3	0	-	0	-	5	6.7
		M+F	150	1	0.7	6	4.0	0	-	0	-	7	4.7

* $p < 0.05$ using χ^2 test. ** $p < 0.01$ using χ^2 test.

TABLE 5. Long-term carcinogenicity bioassay on Mancozeb administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats

HEMOLYMPHORETICULAR NEOPLASIAS AND THEIR DISTRIBUTION BY HISTOCYTOTYPE									
Group No.	Concentration (ppm)	Animals		Animals with hemolymphoreticular neoplasias					
		Sex	No.	Total ^a		Lymphoimmuno-blastic lymphoma ^b		Other lymphomas and leukemias ^b	
				No.	%	No.	%	No.	%
I	1,000	M	75	30	40.0 *	17	56.7	13	43.3
		F	75	16	21.3	11	68.8	5	31.3
		M+F	150	46	30.7	28	60.9	18	39.1
II	500	M	75	35 ^c	46.7 **	24	68.6	13	37.1
		F	75	21	28.0	14	66.7	7	33.3
		M+F	150	56	37.3	38	67.9	20	35.7
III	100	M	75	32	42.7 **	17	53.1	15	46.9
		F	75	27	36.0 **	19	70.4	8	29.6
		M+F	150	59	39.3	36	61.0	23	39.0
IV	10	M	75	22	29.3	16	72.7	6	27.3
		F	75	20	26.7	15	75.0	5	25.0
		M+F	150	42	28.0	31	73.8	11	26.2
V	0	M	75	16	21.3	14	87.5	2	12.5
		F	75	11	14.7	8	72.7	3	27.3
		M+F	150	27	18.0	22	81.5	5	18.5

^aPercentages refer to the number of animals at start. ^bPercentages refer to the number of animals bearing hemolymphoreticular neoplasias. ^cTwo animals bore two different hemolymphoreticular neoplasias. * $p < 0.05$ using χ^2 test. ** $p < 0.01$ using χ^2 test.

at various sites that included malignant mammary tumors, Zymbal gland and ear duct carcinomas, hepatocarcinomas, malignant tumors of the pancreas, malignant tumors of the thyroid gland, osteosarcomas of the bones of the head, and hemolymphoreticular neoplasias.

In the case of follicular tumors of the thyroid gland, a hormonal mechanism may be envisioned (stimulation of the thyrotropic hormone due to the lowering of thyroid hormones produced by ethylenethiourea); this mechanism cannot apply to tumors of other sites.

Our results indicate that Mancozeb should be considered a multipotent carcinogenic agent capable of producing tumors of many types in various sites in treated animals. The results of our study are not consistent with those produced in other laboratories. It should be noted that, in contrast with our study, which continued until the time of spontaneous death of the animals, the bioassays on rats conducted in other laboratories were truncated after 104 weeks of treatment. In our study, most tumors arose after 112 weeks of age. Had we stopped our experiment at 112 weeks of age, it is unlikely that we would have observed the multipotent carcinogenic activity of Mancozeb.¹⁶

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