Effects of Feeding Manganese Antiknock Gasoline Additive Exhaust Residues (Mn_3O_4) in Rats

by J. H. Exon and L. D. Koller

Department of Veterinary Medicine
and the Environmental Health Science Center
Oregon State University
Corvallis, Ore. 97331

Methylcyclopentadienyl manganese tricarbonyl (MMT) is an antiknock additive in unleaded gasoline, and required for use in all automobiles equipped with catalytic converters in the exhaust system. The major residue produced by heating this compound in this manner is hausmanite (Mn_3O_4; manganous manganic oxide), a naturally-occurring form of manganese (ETHYL CORP. 1974). Mn_3O_4 is emitted from automobile exhausts as a potential contaminant of atmospheric, aquatic and terrestrial environments of plants and animals. Possible magnification of manganese residues within food webs could occur due to persistence of manganese in certain animal and plant tissues.

The relative non-toxicity of many manganese compounds to mammals has been reported (GALLUP et al. 1951; RICHARDS 1958; SKINNER 1932; VON OETTINGEN 1935). Conversely, a few manganese compounds have been demonstrated to have very severe effects on experimental animals (CHORNOCK et al. 1942; HYSELL et al. 1974). Methylcyclopentadienyl manganese tricarbonyl produced severe histopathological lesions in lungs, liver and kidneys of rats orally exposed to this compound (HYSELL et al. 1974).

The purpose of this experiment was to observe preliminary clinical and histopathological effects of Mn_3O_4 on laboratory rats and to determine if in-depth toxicological studies of the compound were warranted.

METHODS AND MATERIALS

Twenty, 100 day old male Oregon State University Wistar rats were divided into five groups of four rats each. Rats were fed manganous manganic oxide[1/] (Mn_3O_4) in the feed for 28 days. Group I animals received 2×10^5 ppm Mn_3O_4, group II 2×10^4 ppm, group III 2×10^3 ppm, group IV 2×10^2 ppm and group V served as controls. Rats were housed in pairs in 15"x13"x6½" polycarbonate barrier cages covered with specific pathogen-free filters. Feed

1/ Research Organic/Inorganic Chemical Corp., Sun Valley, CA 91352, 99% Mn_3O_4, 50% Mn.

* This study was supported by USPHS Grants ES-00210 and ES-00040. Technical Paper No. 3956, Oregon Agricultural Experiment Station.

Bulletin of Environmental Contamination & Toxicology, Vol. 14, No. 3 © 1975 by Springer-Verlag New York Inc.

and water were available ad libitum. Each rat was weighed twice a week. Food consumption and signs of clinical abnormalities were monitored daily.

All rats were sacrificed after 28 days on experiment. Sections of tissues (lung, heart, spleen, liver, kidney, brain, adrenal gland, intestine, muscle and spinal chord) were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned, stained with H&E, and examined microscopically for histopathologic changes.

RESULTS

Rats which received 2 x 10^5 ppm Mn_3O_4 (group I) consumed less than one-half as much feed as rats in the other groups (Table 1) and steadily lost weight throughout the experiment.

TABLE 1

Feed and Mn_3O_4 Consumption of Rats Fed Manganous Manganic Oxide for 28 Days

	Feed Consumption (g/kg/day)				Mn_3O_4 Consumption (mg/kg/day)			
	Week No.				Week No.			
	1	2	3	4	1	2	3	4
Group I (2 x 10^5 ppm Mn_3O_4)	9	38	44	45	1750	7500	8730	8900
Group II (2 x 10^4 ppm Mn_3O_4)	33	60	60	54	630	1220	1200	1100
Group III (2 x 10^3 ppm Mn_3O_4)	32	55	61	56	70	110	100	110
Group IV (2 x 10^2 ppm Mn_3O_4	31	58	48	48	4	13	9	9
Group V (Control)	35	59	62	58	-	-	-	-

Rats on all other exposure regimens gained weight during the experimental period, but weight gains decreased as the time of exposure increased when compared to controls (Table 2).

No mortality occurred in control or experimental animals. However, after two weeks on 2 x 10^5 ppm Mn_3O_4, rats in group I were emaciated, lethargic and hypersensitive to handling. After 28 days on experiment, these conditions were more pronounced.

Observations at necropsy for gross pathological changes were negative. Organs and viscera appeared normal except that animals in group I had reduced fat deposits around the kidney and viscera

as compared to rats from the other groups. Histopathology of lung, heart, liver, spleen, kidney, brain, adrenal gland, intestine, and muscle sections did not disclose significant lesions.

TABLE 2

Body Weight and Weight Gain of Rats Fed Manganous Manganic Oxide (Mn_3O_4) in the Feed for 28 Days

	Body Weight (grams)				Weight Gain (g/rat/day)		
	Week No.				Week No.		
	1	2	3	4	1-2	2-3	3-4
Group I (2×10^5 ppm Mn_3O_4)	277	240	216	199	-5.29	-3.43	-2.43
Group II (2×10^4 ppm Mn_3O_4)	293	315	321	324	3.14	0.86	0.43
Group III (2×10^3 ppm Mn_3O_4)	305	317	328	333	1.71	1.57	0.71
Group IV (2×10^2 ppm Mn_3O_4)	318	331	339	341	1.86	1.14	0.29
Group V (Control)	290	305	319	339	2.14	2.00	2.86

DISCUSSION

Previous studies have demonstrated difficulty inducing manganism in experimental animals by oral exposure to manganese compounds (COTZIAS 1958; GALLUP et al. 1951; RICHARDS 1958; SKINNER 1932; VON OETTINGEN 1935). Results of this study were similar in that concentrations of Mn_3O_4 in the feed ranging from 200 to 200,000 ppm were only slightly toxic to rats. Histopathologic observations of selected organs and tissues indicated no evidence of damage by manganese. Clinical signs of manganese toxicity were noted by decreased growth rates of all rats exposed to Mn_3O_4. Growth retardation of several mammalian species following oral exposure to large doses of manganese has been reported previously (COTZIAS 1958; VON OETTINGEN 1932). It is postulated this effect is caused by interference of manganese with normal metabolic pathways of calcium, phosphorous and iron (COTZIAS 1958; CHORNOCK 1942). One study reported a 30% loss of initial body weight of rats after four weeks exposure to high levels of manganese in the feed (CHORNOCK 1942). Effects of feeding high levels of Mn_3O_4 to rats in our experiment were not as severe. Body weights of animals receiving 2×10^2, 2×10^3, and 2×10^4 ppm Mn_3O_4 increased during the four-week exposure period, but growth rates progressively decreased as compared to controls (Table 2). Rats which received 2×10^5 ppm Mn_3O_4 in the feed (group I) lost weight throughout the experiment. This weight loss cannot be attributed entirely to an effect of Mn_3O_4. These animals consumed less than one-half as

much feed as rats on the other experimental regimens, thus magnifying any growth retardation effect of manganese. Decreased food consumption was apparently due to the undesirable flavor imparted to this particular experimental diet by the very high manganese content. The normally white powdered feed was rendered dark gray by the addition of Mn_3O_4. Markedly reduced fat deposits in this group of rats was further evidence of starvation.

It is important to note that animals in this experiment consumed from 4 mg to 8900 mg Mn_3O_4/kg body weight daily (Table 1) for approximately a month without suffering mortalities or apparent tissue damage. Apparently the reaction which occurs from heating methylcyclopentadienyl manganese tricarbonyl, the parent compound initially added to gasoline, reduces the form of manganese to a much less toxic state. The oral LD_{50-14} to rats of the former compound is 58 mg/kg body weight while daily oral doses 150 times greater of Mn_3O_4 were only slightly toxic.

Based on this data, it appears that Mn_3O_4 is a relatively non-toxic compound when consumed and does not warrant extensive investigation as an acutely hazardous environmental contaminant. These data cannot, however, be extrapolated to predict effects of chronic or alternative routes of exposure or to the action of this compound in the presence of additional environmental stresses. Further studies should be done to determine the minimum oral dose required to produce growth retardation. This experiment indicates that this dose is less than 9 mg Mn_3O_4/kg body weight daily.

REFERENCES

COTZIAS, G.C.: Physiol. Rev. 38, 503 (1958).

CHORNOCK, C., GUERRANT, N.B. and DUTCHER, R.A.: J. Nutrition 23, 445 (1942).

ETHYL CORP.: Medical Department, Baton Rouge, LA. Unpublished Report (1974).

GALLUP, W.D., WALTER, L.E. and McOSKER, D.E.: Proc. Oklahoma Acad. Sci. 32, 71 (1951).

HYSELL, D.K., MOORE, W., STARA, J.F., MILLER, R. and CAMPBELL, K.I.: Environ. Res. 7, 158 (1974).

RICHARDS, B.M.: Biochem. J. 24, 1572 (1930).

SKINNER, J.T.: J. Nutrition 5, 451 (1932).

VON OETTINGEN, W.F.: Physiol. Rev. 15, 175 (1935).