The Effects of Dimethylformamide on Female Mongolian Gerbils, *Meriones unguiculatus*

by

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Dimethylformamide has a history as a hepatotoxin, but when small volumes such as 0.1 ml to 0.2 ml were injected into rats no pathological changes were observed (BUTLER 1966). The LD_{50} (intraperitoneal) for dimethylformamide in adult male rats has been reported to be 3,800 (3,000 to 4,600) mg/kg (HEATH 1962). In a separate experiment, an LD_{50} of 1400 mg/kg was reported for adult rats given intraperitoneal injections. The same investigator reported an LD_{50} of 300 mg/kg for mice (MASSMAN 1956).

Acute single dose oral toxicity is of a low order. The approximate lethal dose, administered orally, to rats was reported to be 2,250 mg/kg and 3,400 mg/kg for guinea pigs. Repeated sublethal oral administrations of DMF have given evidence of cumulative toxicity. Rats were reported to have survived 9 to 10 oral doses of 450 mg/kg/day over a period of 11 days, but then weight loss occurred. At the termination of this experiment, some rats showed mild transitory liver damage while others had recovered (DUPONT 1968).

In other studies, dimethylformamide was fed to male and female rats for approximately 90 days at dietary levels of 200, 1,000 and 5,000 ppm. At 5,000 ppm, dimethylformamide caused slight anemia, leukocytosis and hypercholesterolemia. Barely perceptible liver injury was also noted in these animals (DUPONT 1968). In another experiment with mice, rats, guinea pigs and dogs, the major pathological effects of acute administration of dimethylformamide were found to be in the liver (CLAYTON et al. 1963).

Ironically, the use of dimethylformamide as a solvent for other toxins is almost commonplace, especially with the insoluble aflatoxins. The latter group are fungal metabolites that have a high order of acute toxicity in many animal species. In subtoxic quantities they are possibly the most potent hepatocarcinogens known (GOLDBLATT 1969). Such mycotoxins, which may occur in animal feed or in food products consumed by man are soluble in water in the 10 to 30 µg/ml range and slightly more soluble in alcohols. However, for experimental purposes such large volumes of water or alcohol as would be required for dissolving the toxin are not practical. With the use of dimethylformamide it has been found that aflatoxin B_1 is at least soluble in the 20 mg/ml range.

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In a preliminary study we used a DMF-solvent system for administering aflatoxin B_1 to gerbils, *Meriones unguiculatus*. Some toxic responses seen were attributed to the solvent (DUNKIN and LLEWELLYN 1971).

The purpose of this research was to determine the toxic effects of dimethylformamide on *M. unguiculatus* as a preliminary step prior to additional dimethylformamide-aflatoxin studies.

Also additional information may be of value since DMF is widely used as an industrial solvent. For example, in the production of orlon there is a continual concern as to the effect of this solvent on associated workers. Also, there have been no DMF-related toxicological studies utilizing the gerbil as the experimental animal.

Experimental Procedure

Twelve, female Mongolian Gerbils with a mean weight of 50 g were injected subcutaneously (SC) with dimethylformamide (Analytical Reagent; Mallinckrodt Chemical Works, St. Louis, Mo. 63160). The animals were divided into 6 pairs which received injections of 0.10 ml, 0.12 ml, 0.14 ml, 0.16 ml, 0.18 ml, and 0.20 ml. After injection, the animals were placed in individual cages and measurements of their weights were recorded at 72 hour intervals.

Another group of *M. unguiculatus* of similar weight and sex were injected intraperitoneally (IP) with 0.16 ml of dimethylformamide. The animals were then placed in individual cages for observation and recording of their weights. In previous work gerbils had been injected in a range from 0.03 ml to 0.30 ml and we believed 0.16 ml and above to be a lethal dose (DUNKIN and LLEWELLYN 1971).

Sixty gerbils with a weight range of 45 to 110 grams were divided into four experimental groups and one control group. They were housed individually, given standard drinking bottles and laboratory blocks, both *ad libitum*. Dimethylformamide concentrations of 66,000, 34,000, 17,000, and 10,000 ppm in water were used respectively in the four groups. The consumption of the dimethylformamide in respective water solutions as well as the weight of the animals were measured regularly.

A 50 percent solution of dimethylformamide in water was prepared and administered with a #16 oral administration tube, (Phipps and Bird, Richmond, Va. 23220). Intubation with the 50 percent solution was performed on experimental groups in the following ranges; 0.04 ml, 0.08 ml, 0.12 ml, 0.16 ml, 0.20 ml, and 0.24 ml of the solution. The percentage of lethality in each experimental group was then calculated.

Results and Discussion

One of the animals in the group which received an SC injection of 0.10 ml died 3 days after injection, a second animal receiving the same dose died 6 days after the injection and a third animal which had been injected with 0.20 ml died one day after the injection. Time until death ranged from 6 hours to 6 days. The three animals that died had lost about 10 percent of their body weight just before death. These three animals also had the lowest values for their initial body weight. In this section of the study, dimethylformamide caused 25 percent lethality in the SC injection range of 0.10 ml to 0.20 ml. Necrosis was observed at all injection sites. The results are presented in Table 1.

TABLE 1.

Results of Subcutaneous Injection of DMF Given to Female Meriones unguiculatus

Cage Number	Initial Weight (g)	Final Weight (g)	Milliliters Injected	Condition	Time Until Death (days)
1	48.1	43.0	0.10	Died	3
2	47.9	42.7	0.10	Died	6
3	51.0	51.1	0.12	Sacrificed	30
4	51.0	40.5	0.12	Sacrificed	30
5	49.9	51.6	0.14	Sacrificed	30
6	52.5	55.8	0.14	Sacrificed	30
7	50.6	50.5	0.16	Sacrificed	30
8	51.7	56.0	0.16	Sacrificed	30
9	51.1	50.5	0.18	Sacrificed	30
10	52.1	54.2	0.18	Sacrificed	30
11	52.7	49.4	0.20	Sacrificed	30
12	49.3	43.8	0.20	Died	1

IP injections of 0.16 ml of dimethylformamide resulted in 100 percent lethality with time until death ranging from 6 hours to 4 days.

The consumption study using a concentration of 66,000 ppm dimethylformamide in the drinking water yielded an LD_{50} of 3,929 mg/kg over a period of 3 days (LD_{50} based on mean dose consumed by deceased animals). The gerbils that died had a 5 to 10 percent weight loss just before death. The results of this experiment are presented in Table 2.

TABLE 2.

Consumption of a 66,000 ppm Solution of Dimethylformamide in Drinking Water by Female Meriones unguiculatus

Cage Number	Initial Weight (g)	Final Weight (g)	Consumption of Solution (ml)	DMF Consumed (ml)	Time Until Death (days)
1	57.2	48.1	231.1	13.860	22
2	73.8	68.5	4.3	0.258	3
3	67.9	61.0	3.8	0.228	3
4	67.9	64.5	5.9	0.354	3
5	67.9	52.0	79.1	6.086	14
6	62.3	56.6	54.8	3.288	6
7	112.4	106.4	7.4	0.444	3
8	85.5	80.0	20.1	1.206	6
9	112.4	108.9	7.2	0.432	3
10	65.0	61.5	35.4	2.124	8
11	76.9	73.2	4.8	0.288	3
12	61.9	42.3	169.9	10.194	22

Also in the consumption experimental series, the concentration of 34,000 ppm dimethylformamide caused an LD_{50} of 3,846 mg/kg from consumption over a period of 6 days. A consistent weight loss in the animals was not observed in this experiment. The results of this experiment are presented in Table 3.

TABLE 3.

Consumption of a 34,000 ppm Solution of Dimethylformamide in Drinking Water by Female Meriones unguiculatus

Cage Number	Initial Weight (g)	Final Weight (g)	Consumption of Solution (ml)	DMF Consumed (ml)	Time Until Death (days)
1	58.1	57.8	13.9	0.473	3
2	73.4	69.3	169.3	5.756	19
3	53.9	59.7	29.4	0.999	6
4	61.0	62.3	8.5	0.289	3
5	61.8	61.8	6.5	0.221	1
6	73.3	73.3	8.3	0.282	1
7	64.5	64.0	10.1	0.343	3
8	64.5	73.6	87.4	2.971	9
9	66.5	59.2	158.5	5.389	19
10	74.4	73.6	140.9	4.790	9
11	66.0	62.6	6.4	0.218	3
12	71.3	71.3	5.4	0.183	1

Animals in the 17,000 ppm experiment had an LD_{50} of 90,206 mg/kg from consumption over a period of 80 days. No consistent weight loss was observed and a very limited number of necrotic foci were seen in liver tissue.

TABLE 4.

Consumption of a 17,000 ppm Solution of Dimethylformamide in Drinking Water by Female Meriones unguiculatus

Cage Number	Initial Weight (g)	Final Weight (g)	Consumption of Solution (ml)	DMF Consumed (ml)	Time Until Death (days)
1	62.7	56.9	185.4	3.151	27
2	67.8	76.4	651.0	11.067	80
3	62.0	60.6	315.8	5.355	37
4	62.0	67.2	186.9	3.162	22

When the 10,000 ppm dimethylformamide consumption study was terminated after 200 days, the animals had consumed diluted DMF in excess of 100,000 mg/kg body weight. Also, 25 percent of the animals in this group had died at that time. All the livers from the deceased animals had necrotic foci.

In the intubation studies, all of the animals receiving a dose of 0.12 ml or less of the 50 percent DMF solution were alive at the end of the experiment 30 days later. However, 34 percent of the animals receiving 0.16 ml, 45 percent of the animals receiving 0.20 ml, and 67 percent of the animals receiving 0.24 ml died within 24 hours of intubation. The approximate lethal dose appears to be between 0.16 and 0.20 ml.

Our LD_{50} values from the consumption experiments (3,929 and 3,846 mg/kg) fall into the range observed for rats, 3,800 (3,000 to 4,600) mg/kg, which were injected (IP) with DMF (HEATH 1962). The estimated LD_{50} for intubations of a 50 percent solution of DMF can be placed at approximately 3,250 mg/kg which is similar to, but lower than, the preceding values reported in our consumption studies. Our values are higher for the gerbil than those reported for mice (300 mg/kg, IP) indicating that mice may be more sensitive to dimethylformamide (MASSMAN 1956). The weight losses which we observed in the consumption experiment using dimethylformamide at a concentration of 66,000 ppm were also reported for rats given 450 mg/kg/day orally (DUPONT 1968). Our consumption studies and the work of other investigators have shown that quantities of dimethylformamide can be tolerated in low concentrations over extended periods of time but higher concentrations of DMF over a shorter period of time causes toxicity (CLAYTON et al.

1963). This was true with the exception of a few animals in each of the drinking water-DMF experiments where there were always one or two surviving animals which had consumed sufficient quantities of DMF to cause death. Our only explanation is that of varying sensitivity from animal to animal.

Essentially the same liver pathology was observed in the animals receiving SC injections, IP injections and animals in the consumption experiments of 66,000 ppm, 34,000 and 17,000 ppm. Liver lesions were produced in nearly all of the experimental animals. These lesions included zones of diffuse necrosis, hyperchromatic nuclei, abnormal numbers of mitotic figures, giant nuclei and hemosiderin. Many Kupffer cells were present in the liver tissue indicating degeneration of that tissue. Both gross and microscopic pathology were present in some cases. There was a direct relationship between the quantity of DMF consumed or injected and the number of necrotic foci found in the liver. Levels of DMF that caused rapid loss of body weight prior to death also appeared to cause histopathological liver lesions. In general, the resulting gerbil pathology was that of toxic hepatitis and congested kidneys. This agrees with other studies using mice, rats, guinea pigs and dogs which also showed that the main pathological effect of dimethylformamide was on the liver (CLAYTON et al. 1963).

Summary

The female Mongolian Gerbil does not appear to be highly sensitive to DMF when compared to other species. Induced liver pathology and changes in body weight were similar irrespective of the route of administration. The toxic response from a single administration, as noted by the LD_{50}, appears to be within the 3,000 to 4,000 mg/kg for IP, SC and intubated quantities. Consumption studies had the following LD_{50} values for related toxin levels: 66,000 ppm, 3,929 mg/kg in 3 days; 34,000 ppm, 3,846 mg/kg in 6 days; 17,000 ppm, 90,206 mg/kg in 80 days and 10,000 ppm, more than 100,000 mg/kg in more than 200 days.

DMF imbibed at 10,000 ppm for 30 days in drinking water failed to cause observable liver, kidney or weight changes. Higher dosage levels of DMF, as reported, did cause death and physiological changes. DMF levels causing loss of weight were also causing serious pathological changes in the liver or kidneys in the form of necrotic foci. This loss of weight was generally associated with acute toxicity.

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