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## SHORT PAPERS

# Reproduction Study with Formaldehyde and Hexamethylenetetramine in Beagle Dogs

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**Summary**—The effects of hexamethylenetetramine (HMT) and formaldehyde (FA) on reproduction were studied in 51 beagle bitches (mean body weight 12 kg). The dogs were fed on days 4-56 after mating with a daily ration of 300 g dry pellets containing either FA or HMT at levels of 125 or 375 ppm FA or 600 or 1250 ppm HMT. Control animals were given untreated pellets. The treatments did not affect the pregnancy rate, the weight gain of the pregnant beagles, the length of gestation or the size of the 44 litters. The percentage of still-born pups showed a slight increase and the weight gain of the pups and the survival to weaning were slightly impaired by the higher dietary level of HMT. No malformations (either internal or skeletal) were observed in any of the 264 live-born and 20 still-born pups.

### Introduction

The use of hexamethylenetetramine (HMT) as an antimicrobial agent in food has caused concern, mainly because of a lack of toxicological data and a suspected carcinogenic action (Joint FAO/WHO Expert Committee on Food Additives, 1962). Subsequent short- and long-term studies revealed a very low toxicity and no evidence of carcinogenicity in mice and rats (Brendel, 1964; Della Porta, Colnaghi & Parmiani, 1968 & 1970; Natvig, Andersen & Wulff Rasmussen, 1971). Two reproduction studies in the rat showed no effect on fertility or the incidence of abnormalities in the litters (Della Porta, Cabral & Parmiani, 1970; Natvig *et al.* 1971), but no reproduction studies in dogs as requested by the Joint FAO/WHO Expert Committee on Food Additives (1967) have been published.

The present study was undertaken to investigate the effects of the oral administration of HMT to beagle dogs during gestation. As the toxicological effects of HMT appear to be due to the liberation of formaldehyde (FA), other groups of animals were treated with FA.

### Experimental

**Animals.** The 51 bitches used were from our closed breeding colony of beagle dogs and had already successfully raised one or more litters. When 9 wk old, all animals had had a combined vaccination against distemper, hepatitis and leptospiroses, and a booster vaccination was given yearly. A dose of piperazine citrate was administered at 4 and 6 wk of age as well as 4 and 6 wk after each mating. Breeding bitches were housed in groups of four or five in indoor cubicles connected to an outdoor run, each pregnant bitch being transferred to an air-conditioned whelping room 1 wk before term.

**Diet.** The dogs were fed exclusively on dog pellets (Nafag AG, Gossau SG, Switzerland) and were fasted each Saturday. The daily ration for bitches was 300 g, which was reduced



to 200 g 1 wk before term. No food was offered on the day of parturition. The animals were given 300 g on the following day, and 300 g twice daily for the next 3 wk. From wk 4 to the end of wk 12, the mother was allowed 250 g twice daily, while the pups in temporary isolation received two feeds of 150 g daily. Pups of inadequately lactating mothers were supported with reconstituted cows' milk.

*Experimental design and conduct.* Commercial grade HMT and FA were used, FA being provided as a 40% solution. HMT was given at dietary levels of 600 and 1250 ppm and FA at dietary levels of 125 and 375 ppm. Solutions containing the required daily dosage in 2.0 ml were prepared weekly and sprayed on the pellets prior to feeding. The pellets were promptly consumed (within 5–10 min) before the FA had a chance to volatilize. The treatment was initiated 4 days after mating and lasted until day 56, at which time the dog was transferred to the whelping room. The dosage regimes and group sizes are detailed in Table 1. The bitches were weighed at weekly intervals throughout pregnancy and lactation. The pups were weighed at birth and twice weekly thereafter. They were inspected for visible defects immediately after birth and after 8 wk. Stillborn pups and those lost before weaning were autopsied and examined for internal and skeletal abnormalities.

Table 1. Dosage schedules and fertility of female beagles fed diets containing FA or HMT during gestation

Diet	Treatment	Dietary level (ppm)	Dose (mg/kg body weight/day)	No. of mated bitches	No. of pregnant bitches	No. of litters	Mean length of gestation (days)
Control		0.0	0.0	11	9	9	65.8
FA		125	3.1	11	10	10	63.6
		375	9.4	10	9	9	64.7
HMT		600	15	9	8	8	63.3
		1250	31	10	9	8	63.5

*Further observation.* Of the pups weaned in this experiment, it was possible to observe 212 for longer periods. Two dogs were observed for 5 months, 36 for 6, 81 for 7, 64 for 8 and 11 for 9 months, while the remaining 18 dogs were used for breeding purposes and are still in our beagle colony.

#### Results and Discussion

The treatments did not affect the pregnancy rate (Table 1). One pregnant bitch in the group given the higher level of HMT was severely injured in a fight and had to be eliminated. The body weight increased regularly during pregnancy in all groups and the duration of gestation was unaffected by the treatments (Table 1).

Table 2 presents the mean litter sizes in the various test and control groups, as well as the mean numbers of pups of each sex born live and weaned. The mean litter size was within the normal range for all groups, demonstrating that fecundity was not affected by treatment. In the group that received the higher dose of HMT, the percentage of stillborn pups was higher than in any of the other groups, mainly because in one litter of nine pups only two were born alive. No skeletal or any other malformations were observed in any of the stillborn pups.

Table 2. Fecundity of female beagles given FA or HMT orally during gestation

Diet (ppm)	No. of litters	Total pups at birth	Total live pups at birth	Total pups at weaning	Mean pups/litter					
					Total births		Live births		At weaning	
					Male	Female	Male	Female	Male	Female
Control	9	60	56	49	3.1	3.6	2.9	3.3	2.3	3.1
FA (125)	10	54	50	50	2.6	2.8	2.4	2.6	2.4	2.6
FA (375)	9	64	64	60	4.2	2.9	4.2	2.9	3.9	2.8
HMT (600)	8	50	48	45	3.3	3.0	3.3	2.8	3.1	2.5
HMT (1250)	8	56	46	33	3.7	3.3	2.6	3.1	1.5	2.6

Data on the body weight of the pups is summarized in Table 3. During the first month there was a retardation of growth in the group given the higher dose of HMT, coinciding with an increase in mortality. In the same group the percentage of pups that survived to weaning was lower than in the other groups.

Table 3. Mean body weight of pups born to beagles given FA or HMT orally during gestation

Diet (ppm)	Mean body weight (g) of pups* at wk								
	0†	1	2	3	4	5	6	7	8
Control	321	547	818	1078	1264	1601	2020	2449	2957
FA (125)	308	526	755	987	1247	1512	1816	2263	2712
FA (375)	294	467	706	944	1166	1429	1741	2145	2587
HMT (600)	290	508	753	1046	1295	1499	1805	2324	2780
HMT (1250)	294	421	648	889	1125	1425	1742	2160	2698

\*In each group values are means for all the pups surviving at the time stated.

†Weight at birth.

All the dogs observed for a more prolonged period have been normal in behaviour, appearance, motility and muscular co-ordination. The dogs observed up to 9 months were used for various other investigations, for which they were eventually killed and autopsied. No malformations were found. The 18 dogs transferred to the breeding colony have been under observation for nearly 2 yr. Neither these adults nor their litters have shown any signs of physiological or skeletal abnormalities or disorders of reproduction.

The present study thus revealed no teratogenic action of FA or HMT. Even with the higher dose of HMT given, corresponding to a daily intake of 0.031 g/kg body weight, the adverse effect on reproduction was slight.

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the mean numbers of pups of this sex were

the normal range for all groups, and were

In the group that received the highest dose

higher than in any of the other groups.