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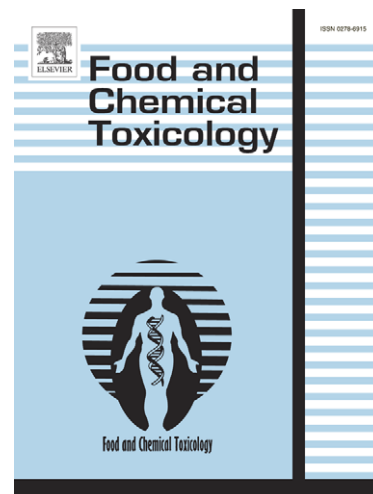
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Oral Developmental Toxicity Study of Methylsulfonylmethane in Rats

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¹ Abbreviations: MSM = Methylsulfonylmethane; NOAEL = no-observed-adverse-effect level

ABSTRACT

Methylsulfonylmethane (MSM) is a metabolite of dimethyl sulfoxide, and occurs naturally at low levels in many foods. MSM has received wide attention as a dietary supplement to promote joint health. The objective of these studies was to determine the developmental toxicity potential of MSM when administered orally to pregnant rats during the period of major organogenesis and histogenesis. In a preliminary dose-finding study, distilled MSM microprill (*i.e.*, microspherical pellets of MSM) was administered by oral gavage at dose levels of 0 (vehicle control), 50, 250, 500, and 1000 mg/kg/day to 8-9 sperm-positive female Sprague-Dawley rats/group/day on gestation days 6 through 20. No evidence of maternal or fetal toxicity was observed. For the definitive developmental study, four groups of 24-25 timed-bred primiparous female rats were administered 0, 50, 500, or 1000 mg MSM/kg/day *via* gavage on gestation days 6 through 20. Maternal feed consumption, body weight, body weight gain, uterus weight and corrected body weight/body weight gain were unaffected by treatment. No evidence of maternal toxicity, and no significant differences in litter viability, litter size, or litter body weight were detected. Fetal evaluations failed to show any biologically significant increase in the incidence of anomalies in the MSM treated groups, and no malformations were seen in any of the fetuses. No evidence of fetal mortality, alterations to growth, or structural alterations were observed in the fetuses of dams administered 50 to 1000 mg/kg/day. Therefore, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 1000 mg/kg/day.

1. INTRODUCTION

Methylsulfonylmethane (MSM) is an organic sulfur-containing compound that occurs naturally in a variety of foods including fruits, vegetables, grains, and beverages (Pearson *et al.*, 1981; Silva Ferreira *et al.*, 2003). MSM has also been detected in tissues of animals (Imanaka *et al.*, 1985) and humans (Williams *et al.*, 1966; Engelke *et al.*, 2005). MSM is considered a source of sulfur for cysteine and methionine production (Richmond, 1986). In recent years, MSM has been extensively used as a dietary supplement for its potential to improve human health, and to reduce arthritic and rheumatic pain (Hasegawa *et al.*, 2004; Kim *et al.*, 2006).

The safety of MSM has been evaluated in a number of rodent studies. In a subchronic study, male and female Wistar [CrI:(WI)BR] rats (20/sex) were orally administered (*via gavage*) either distilled water or 1500 mg/kg/day of MSM in distilled water for 90 days (Horváth *et al.*, 2002). The major organs were weighed and fixed in formaldehyde for histopathological observations, and clinical chemistry parameters (*i.e.*, serum enzymes, lipid profile, serum proteins, albumin and blood chemistries) and urinalysis parameters (*i.e.*, appearance, volume, specific gravity, pH, protein, glucose, and blood) were analyzed. No effects on body weight, hematological parameters or histopathological lesions in organs and tissues were found. At necropsy, no gross pathological changes or differences in organ weights were noted, except for a significant increase in kidney weights of treated male rats. Histopathological examination of kidneys in both males and females did not reveal any treatment related lesions, and therefore the significant kidney weight difference was considered by the authors as likely due to low within-group deviations rather than a toxicological effect.

We recently evaluated the absorption, distribution and excretion of orally administered MSM in rats, and found that [³⁵S]-MSM is rapidly absorbed, well distributed, and efficiently excreted from the body (Magnuson *et al.*, 2006). These data are in agreement with an earlier report of the rapid elimination of MSM (Otsuki *et al.*, 2002). Recently, Kim *et al.* (2006) reported no difference in the incidence of minor adverse effects in arthritic patients consuming either placebo or 6g MSM orally/day for twelve weeks.

The effect of oral MSM exposure on mammalian development has not yet been fully investigated. In a gametogenesis study, Goldstein *et al.* (1992) used the nematode, *Caenorhabditis elegans*, as a model to investigate the effects of MSM on developmental and reproductive genetics. In that study, *C. elegans* ($n=3$ worms/concentration) were exposed to MSM at concentrations of 0.5, 1, 2, and 5% for eleven days, which represents approximately half the lifespan of *C. elegans*. Fecundity, viability and morphology of worms were measured. MSM had little effect on the morphology of worm offspring at a concentration of 0.5%. At higher concentrations, loss of viability and fertility and a delay in development were noted. The observed decrease in life span of the nematodes was associated with senescent morphology of meiotic prophase nuclei, such that nuclei from young and old specimens were indistinguishable. At MSM concentrations >0.5%, synaptonemal complexes were absent from pachytene nuclei, prohibiting the effective pairing and segregation of homologous chromosomes. Effects on X-chromosome nondisjunction were reported; however, no data on the concentration needed to induce this effect was provided (Goldstein *et al.*, 1992). Although the results of this *in vitro* investigation in nematodes may indicate developmental effects of MSM, it is difficult to extrapolate results from nematodes to mammals. Thus, the present studies were undertaken to

determine the developmental toxicity potential of MSM when administered orally to rats during the period of major organogenesis and histogenesis.

2. MATERIALS AND METHODS

2.1. Test Animals

Male and female Sprague-Dawley rats [NTac:(SD)], (Taconic Farms, Germantown, NY), approximately seven to nine weeks of age and weighing between 145 – 215 g/rat, were held in quarantine in plastic cages at $22\pm 4^{\circ}\text{C}$ with 12-hour light/dark cycle for approximately two weeks prior to the studies, during which time they were observed daily for survival. Throughout the study, Harlan Teklad Certified Rodent Diet #8728C and drinking water were supplied *ad libitum*. During the quarantine period, vaginal smears were collected over at least three consecutive days prior to mating to ensure cyclicity of the females and later to determine the stage of the estrus cycle for mating purposes. Rats were mated overnight in a 1:1 mating scheme (*i.e.*, one male:one female); mating was confirmed by the presence of sperm in the vaginal smear. Upon evidence of a vaginal plug indicating mating had occurred, males were removed and females were individually housed for the rest of the experiment. The studies were conducted in accordance with U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations.

2.2. Test Material

The test material used in this study was OptiMSM[®] distilled microprill² (CAS registry number 67-71-0) provided by Cardinal Nutrition, Inc. (Vancouver, WA). Formulations were prepared in deionized water at 10, 100, and 200 mg/ml concentrations for the 50, 500, and 1000 mg/kg dose levels, respectively. Aliquots of the formulations were transferred to dosing vials and

² MSM converted into solid microspherical pellets by spray formation.

samples representing each dose level from the first preparation were analyzed by gas chromatography. The purity of MSM was 99.9% and the formulations were stable for 32 days for concentrations ranging from 10 to 100 mg/ml when stored at room temperature.

2.3. Preliminary teratogenicity dose range-finding study

A preliminary teratogenicity study was conducted to determine the dose for the definitive teratogenicity study. Approximately 48 nulliparous Sprague-Dawley female rats were mated to attain a minimum of 42 pregnant rats. Five groups with 8-9 timed-bred primiparous dams *per* group were administered 0 (vehicle only), 50, 250, 500, or 1000 mg MSM/kg *via* oral intubation. Dosing occurred daily on gestation days 6 through 20, which included the period from implantation to closure of the hard palate and histogenesis. Maternal body weight, body weight gain and feed consumption were measured throughout gestation. Dams were euthanized on their respective 21st day of gestation and subjected to gross necropsy and caesarean section. The uteri were weighed, opened and inspected for implantation sites; fetuses were harvested, weighed and given a gross external examination.

2.4. OECD guideline teratogenicity study

The definitive teratogenicity study was conducted according to US Food and Drug Administration principles (FDA, 1982) and Organization for Economic Co-operation and Development (OECD) guidelines for developmental toxicity (No. 414) (OECD, 2006). Approximately 145 nulliparous rats were mated to attain a minimum of 80 pregnant rats. The study consisted of four groups of 24-25 timed-bred primiparous dams/group. Rats were administered 0 (vehicle only), 50, 500, or 1000 mg MSM/kg *via* oral intubation at a constant volume of 5 ml/kg. Dosing occurred daily on gestation days 6 through 20. To facilitate the teratogenic evaluation of pups, a staggered-start design (*via* staggered mating) was employed.

Mating was staggered over six days resulting in six series. Dams determined as having mated for a given series were randomly assigned to the study groups, based on body weight measured on day zero. Each series was euthanized on its respective 21st day of gestation. Body weights were recorded on gestation days 0 (sperm-positive day/randomization), 3, 6, 9, 12, 15, 18, and 21. Feed consumption was calculated for each period between weighings.

All dams were euthanized using CO₂ asphyxiation on their 21st day of gestation and underwent a cesarean section. Gross necropsy consisted of examination of the brain and all organs in the thoracic and abdominal cavities of the dams. Pregnancy status, number of corpora lutea, number and distribution of live fetuses and embryonic/fetal deaths, individual pup weights, and sex were recorded for each dam. The sex of the fetus was determined as part of the gross external examination. The uteri of dams that appeared to be non-gravid were stained with 10% ammonium sulfide and examined. The uterus and ovaries were weighed. Uterine weights collected from non-gravid animals were excluded from calculations.

Fetuses were given a gross external morphological examination consisting of: evaluation of the shape of the body and head, size and extension of the limbs, enumeration of all separate digits, inspection of the skin, umbilicus region, anus and genitals, as well as inspection of the nares, pinna, eyes, and oral cavity was conducted on each fetus. All abnormalities were recorded. Each uterine horn was inspected for tissue resorptions and fetal deaths. Implantation sites were counted, recorded and classified as: early resorption (placenta only); late resorption (placenta and fetal remains); early death (fetus weight less than 0.8 g); and late death (fetus weight of more than 0.8 g). Fetuses were euthanized by the induction of hypothermia. One-half of the fetuses from each litter were randomly assigned to receive either a skeletal examination or were decapitated and

subjected to a visceral and cephalic examination. For fetuses designated for visceral examination, external and internal sex was determined.

For skeletal examinations, fetuses were fixed in alcohol and stained with Alizarin red/potassium hydroxide solution. Skeletal anomalies were scored as follows: 0, no visible abnormality; 1, variation within normal limits; 2, slight variation; 3, moderate variation; 4, severe variation; and 5, malformation. The bones evaluated for anomalies are presented in Table 1.

<Insert Table 1>

Visceral examinations were performed as previously described (Stuckhardt and Poppe, 1984). Cephalic examinations were performed on decapitated fetal heads fixed in Bouin's solution using a modified method of Wilson's razor blade sectioning technique (Taylor, 1986). Examinations were done on the control and high dose groups, and included: palate and upper lip, nasal septum, olfactory lobes of the brain, ventricles I, II and III of the brain, optic cup, retina, lens and cornea.

2.5. Statistics

Dam weights, litter body weights, and viability data were analyzed by a two-way analysis of variance (ANOVA). In the presence of a significant main effect, all post-hoc comparisons were performed using Dunnett's test (two-tail). Gross, visceral, cephalic, and skeletal data were analyzed by Chi-Square/Fisher's Exact tests (fetal N) when incidence in the treated rats was higher (*i.e.*, when the difference in the absolute number of fetuses affected is greater than three) than controls. A minimum statistical significance level of $p \leq 0.05$ was used in all cases.

3. RESULTS

3.1. Preliminary study

Pregnancy rates were 100% in the control group and all treatment groups (except the low dose group which had pregnancy rate of 87.5%), from the 7-9 gravid rats/group (Table 2). Litter size, litter weight, number of resorptions and fetal deaths were similar across all groups (Table 2). Male-to-female fetal ratios were similar across groups (Table 2). No evidence of developmental toxicity, developmental delay or gross external malformations was observed in fetuses of dams dosed with MSM up to 1000 mg/kg body weight/day. No evidence of maternal or fetal toxicity was observed. Maternal body weight, uterus weight, body weight gain and feed consumption were not adversely affected by treatment (data not shown). Thus, the no-observed-adverse-effect level (NOAEL) for maternal and fetal toxicity of MSM was greater than 1000 mg/kg/day when administered during gestation days 6 through 20 in the preliminary study.

<Insert table 2 here>

3.2. Definitive study

Maternal health parameters

Maternal body weight was similar among all groups (Figure 1). Maternal feed consumption, body weight gain and total gain were unaffected by MSM (data not shown). Uterine weights and body weights, corrected for uterine weight, were similar across all groups. Dams appeared normal throughout the study and no clinical signs of toxicity were observed. At necropsy, one control dam had a cyst on its ovary, and one mid dose dam had an inguinal mass. No evidence of maternal toxicity by MSM was observed in this study.

<Insert figure 1 here>

Litter Viability

Successful pregnancy ranged from 80 to 100% resulting in 20-24 gravid rats/group, each with viable litters (Table 3). No statistically significant differences were detected in the number of live or total implants, resorptions, corpora lutea, or *percent* pre- or post- implantation loss (Table 3). One dead fetus was observed in the low dose group, which was not considered an adverse or treatment-related effect.

<Insert table 3 here>

Fetal Weights and Gross External Morphology

No significant differences in male, female or combined fetal weights were detected (Table 4). Male-to-female fetal ratios were similar across groups (1.2:1 in the control and low dose groups, 1.1:1 in the mid dose, and 1:1 in the high dose group) (Table 4). No gross external anomalies were observed; all fetuses appeared normal and none were malformed (Table 5). One fetus in the low dose group had a red mark on its neck; this was related to removal of the fetus from the uterus and was not considered an adverse or treatment-related finding.

<Insert table 4 here>

Visceral Examinations

No visceral malformations were observed in any of the fetuses examined (Table 5). Visceral anomalies consisting principally of hydroureter and/or hydronephrosis of slight severity were seen at a low incidence (1-3 fetuses/group) across all groups, including controls. In addition, one high dose fetus had a reversed umbilical artery coursing along the bladder (an anatomical variation considered a normal occurrence in the Sprague-Dawley strain of rat).

<Insert table 5 here>

Cephalic Examinations

No abnormalities were seen in the control or high dose fetuses (Table 5). Fetal cephalic exams were unremarkable and provided no evidence that MSM produced any adverse or teratogenic effect in the offspring.

Skeletal Examinations

No treatment-related skeletal anomalies were seen in the low, mid or high dose groups (Table 6). Common skeletal variations (rudimentary 14th rib, incomplete ossification of the skull, wavy ribs, extra ossification site along the vertebral column, and misshapen centrae) were noted in all groups at a similar incidence with the exception of ossification of the skull (Table 7). The overall variation noted was incomplete ossification in the skull, with the number of bones/bone pairs affected ranging from 1 to 3. This could be due to incomplete ossification of the parietal, interparietal, and superoccipital bones. Specifically, the incidence of incomplete ossification of the interparietal bone of the skull was significantly greater in the high dose fetuses compared to controls. Single bones displaying incomplete ossification at the level of “normal/within normal limits” receive a grade score of 1 and, it is possible to have a higher incidence of incomplete bone ossification but not be considered a malformation or a teratologic change. In addition, although the incidence in the high dose fetuses was above the concurrent control, it was within range when compared to in-house historical controls (22%). The thoracic/lumbar skeletal regions were evaluated, with dumbbell, bipartite centers of the vertebral column commonly seen in both treatment and control dose groups. In typical teratological evaluations in rats, one center of the vertebral column has been considered as within normal limits, with two and three centers thought

of as “more” severe, although two and three centers are variations seen at a low background incidence. A number of variations were observed in the fifth and sixth centers of the sternum, but were considered normal. The number of fetuses without any skeletal variations (% Normal) was highest in the low dose group (74%), followed by the mid dose group (71%); the control and high dose groups were similar with the number of fetuses not displaying any variation being 67 and 63%, respectively (Table 6).

<Insert table 6 here>

Embryo-Fetal Toxicity Summary

No evidence of embryo or fetal toxicity, or treatment-related alterations in fetal body weights or fetal examinations (gross external, visceral, cephalic, or skeletal) was observed in this study at doses up to 1000 mg/kg MSM.

<Insert table 7 here>

4. DISCUSSION

These studies are the first report evaluating the developmental toxicity of MSM in a mammalian model. We observed no evidence of developmental toxicity of MSM when administered orally to rats during the period of major organogenesis and histogenesis at doses up to 1000 mg/kg/day.

The findings from a *C. elegans* study (Goldstein *et al.*, 1992) in which nematodes exposed to MSM displayed a dose-dependent loss of viability and fertility raised the question of potential developmental toxicity of MSM. However, the lowest concentration tested was 0.5% of the culture media. At MSM concentrations greater than 0.5%, electron microscopy images of the

treated worms detected the absence of synaptonemal complexes, which would prohibit the effective pairing and segregation of homologous chromosomes. The relevance of this finding of an effect of exposure of *C. elegans* to culture media containing greater than 0.5% MSM, to a potential adverse effect due to oral consumption of MSM, the major route of exposure for humans, is difficult to assess.

The genotoxicity of MSM was recently evaluated using *in vitro* and *in vivo* assays (Lee *et al.*, 2006). MSM did not induce revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1538 at concentrations up to 10,000 µg/plate with or without S9 fractions. No evidence of the ability of MSM to induce chromosomal damage was observed from *in vitro* chromosomal aberration assays with CHL cells with or without S9 fractions treated with concentrations up to 5000 µg MSM/ml, which is in disagreement with the results by Goldstein *et al.* (1992). In the same study (Lee *et al.*, 2006), groups of six mice were given a single dose of MSM (0, 1250, 2500, and 5000 mg/kg) or 2 mg/kg mitomycin C *i.p.* (positive control). Bone marrow smears were prepared 48 hours after dose administration and evaluated for micronucleus aberrations. A significantly higher number of micronucleated polychromatic erythrocytes were observed in smears from mice treated with mitomycin C compared to control mice, but there was no difference in the number of micronucleated polychromatic erythrocytes in MSM-treated mice compared to control mice. Therefore, no evidence of genotoxicity was observed in the bone marrow of MSM-treated mice up to 5000 mg/kg.

The results of the current developmental study in rats supports the work by Lee *et al.* (2006), as no evidence of treatment-related visceral or cephalic teratogenic malformations were observed at doses up to 1000 mg MSM/kg/day. Although the current study indicated variations in the skeletal parameters of the fetuses, these variations were considered within normal ranges for

this strain of rats. Sternebral ossification may vary, when evaluating the uniformity of the sternebral centers. In general, the first through fourth sternebral centers are consistently uniform in ossification, size, and shape, while the fifth and sixth centers can be incompletely ossified or small and still be considered normal (Taylor, 1986). All structures are not completely formed/ossified on gestation day 21, and the time of mating/implantation can vary by a few hours between animals, resulting in a normal variation in ossification. Goldstein *et al.* (1992) noted reduced viability and fertility in nematodes subjected to MSM *in vitro*, while the current results indicate that MSM administered orally to rats does not affect viability or fertility at doses up to 1000 mg/kg/day during the critical period of organogenesis.

Our findings of no effect of doses up to 1000 mg/kg/day in the dams in this study also support previous results by Horváth *et al.* (2002), who found that MSM administered for 90 days to rats at 1500 mg/kg/day did not cause any mortality, or treatment-related changes in clinical signs, body weight or food consumption. Based on the Horváth *et al.* (2002) study, a subchronic NOAEL for MSM of 1500 mg/kg/day can be established.

In summary, oral administration of MSM to pregnant rats at doses of 50, 500, or 1000 mg/kg/day over gestation days 6-20 (the period of organogenesis and histogenesis) did not result in any biologically significant alterations in the fetal or maternal body weights, nor in any structural malformations or fetal anomalies as evaluated by gross external, cephalic, visceral and skeletal examinations. Our results indicate that the NOAEL for maternal and developmental toxicity of oral MSM is at least 1000 mg/kg/day. These data support the mounting evidence of safety-in-use of MSM.

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Footnotes

¹ MSM = Methylsulfonylmethane; NOAEL = no-observed-adverse-effect level
In page 1

¹ MSM converted into solid microspherical pellets by spray formation.
In page 5

Table 1. Skeletal formations examined for malformations.

Skull	Clavicles	Pelvic Girdle
Nasals	Complete Vertebral Column	Ilia
Frontals	Centra	Ischia
Parietals	Arches	Pubes
Interparietal	Ribs	Scapulae
Supraoccipital	Forelimbs	Sternebrae
Exoccipital	Humeri	
Premaxillae	Radii	
Maxillae	Ulnae	
Mandibles	Metacarpals	
Zygomastics	Phalanges	
Squamosals	Hindlimbs	
Tympanic annuli	Femora	
Presphenoid	Tibiae	
Basisphenoid	Fibulae	
Basioccipital	Metatarsals	
Hyoid Process	Phalanges	

Table 2. Pregnancy and embryo-fetal data in Sprague-Dawley rats administered MSM orally from gestation days 6 to 20 (preliminary study)

	Treatment Group				
	Vehicle Control	Low 50 mg/kg/day	Mid 250 mg/kg/day	Mid-High 500 mg/kg/day	High 1000 mg/kg day
Dams Entering Study (sperm-positive)	9	8	8	8	9
% Successful Pregnancy (viable)	100	87.5	100	100	100
Viable Litters (at least 1 live implant)	9	7	8	7	9
Corpora Lutea/Animal	15 ± 1 ^a	16 ± 1	15 ± 1	14 ± 1	16 ± 1
Total Implants	13 ± 1	14 ± 2	14 ± 1	13 ± 1	15 ± 1
Live Implants	12 ± 1	14 ± 2	14 ± 1	13 ± 1	15 ± 1
Non-live Implants (Resorptions)	1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Total Number of Male Fetuses	6 ± 1	9 ± 1	6 ± 1	7 ± 1	8 ± 1
Total Number of Female Fetuses	7 ± 1	7 ± 1	8 ± 1	6 ± 0	7 ± 1
Male:Female Ratio	1:1.1	1.3:1	1:1.3	1.3:1	1.1:1
Malformed Fetuses	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean Fetal Body Weight (g)					
Males	5.8 ± 0.1	5.5 ± 0.1	5.9 ± 0.1	5.5 ± 0.1	5.6 ± 0.1
Females	5.6 ± 0.1	5.2 ± 0.1	5.6 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
Combined	5.7 ± 0.1	5.4 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.5 ± 0.1

^aMean ± S.E

Table 3. Pregnancy and embryo-fetal data in Sprague-Dawley rats administered MSM orally from gestation days 6 to 20

	Treatment Group			
	Vehicle Control	Low 50 mg/kg	Mid 500 mg/kg	High 1000 mg/kg
Initial Group Size (sperm positive)	25	24	25	24
Actual Group Size (gravid)	20	24	23	23
% Successful Pregnancy	80.0	100.0	92.0	95.8
Viable Litters (at least 1 live implant)	20	24	23	23
Litters with Resorptions	8	8	4	7
% Litters with Resorptions	40.0	33.3	17.4	30.4
Litters with Deaths	0	1	0	0
Litters with Malformations	0	0	0	0
Litters with Resorptions and Deaths	0	1	0	0
% Pre-Implantation Loss ^{a,b}	8.7±2.4	10.6±3.0	11.9±3.4	10.5±2.4
% Post-Implantation Loss ^{b,c}	5.0±1.8	5.2±1.8	1.3±0.6	5.5±3.0
Corpora Lutea ^b	14.1±0.4	13.6±0.5	14.1±0.4	14.2±0.4
Live Implants ^b	12.3±0.5	11.6±0.5	12.5±0.6	11.9±0.6
Total Implants ^b	12.9±0.5	12.2±0.5	12.7±0.7	12.7±0.5
Nonlive Implants ^d	0.6±0.2	0.6±0.2	0.2±0.08	0.8±0.2
Live Fetuses/Total Implants	245/257	278/292	287/291	274/292
% Live Fetuses/Total Implants	95	95	99	94
Nonlive Implants/Total Implants	12/257	14/292	4/291	18/292
% Nonlive Implants/Total Implants	4.7	4.8	1.4	6.2
Malformed/Live Fetuses ^b	0	0	0	0
% Malformed/Live Fetuses	0	0	0	0
Nonlive and Malformed/Total Implants	12/257	14/292	4/291	18/292
% Nonlive and Malformed/Total Implants	4.7	4.8	1.4	6.2

^a % Pre-implantation Loss = [(Total Corpora Lutea - Total Implants) ÷ Total Corpora Lutea] * 100

^b The mean reported utilizes the litter as the unit of observation ± S.E.; the N used is the number gravid unless the parameter requires an intact fetus for evaluation (e.g., malformed), in which case the N utilized is viable litters

^c % Post-implantation Loss = [(Total Implants - Live) ÷ Total Implants] * 100

^d Non-live implants includes resorptions and deaths

ANOVA, $p > 0.05$

Table 4. Litter body weight and fetuses in Sprague-Dawley rats administered MSM orally from gestation days 6 to 20

		Treatment Group			
		Vehicle Control	Low 50 mg/kg	Mid 500 mg/kg	High 1000 mg/kg
Group Size (gravid)		20	24	23	23
Litter Weight (g)					
Males		5.81±0.08 ^a	5.92±0.05	5.70±0.09	5.70±0.1
Females		5.50±0.07	5.65±0.04	5.44±0.08	5.49±0.09
Combined		5.66±0.08	5.79±0.04	5.57±0.08	5.60±0.09
Number of Fetuses					
Males	Sum	132	152	149	140
	Mean	6.60±0.5	6.33±0.5	6.48±0.5	6.09±0.5
Females	Sum	113	127	138	134
	Mean	5.65±0.5	5.29±0.5	6.00±0.5	5.83±0.4
Combined	Sum	245	279	287	274
	Mean	12.25±0.5	11.63±0.5	12.48±0.7	11.91±0.6
Male:Female Ratio		1.2:1	1.2:1	1.1:1	1:1

^aMean ± S.EANOVA, $p > 0.05$

Table 5. Gross external morphology, visceral and cephalic observations in fetuses from Sprague-Dawley rats administered MSM orally from gestation days 6 to 20

	Treatment Group			
	Vehicle Control	Low 50 mg/kg	Mid 500 mg/kg	High 1000 mg/kg
<u>Gross External Morphology</u>	245/20 ^a	279/24	287/23	274/23
Variation	-/ ^b	-/	-/	-/
Minor Observations^c	-/	1/1	-/	-/
Red mark on neck	-/	1/1	-/	-/
<u>Visceral Examination</u>	123/20	138/24	142/23	136/23
Variation	1/1	2/2	2/2	4/4
Slight hydroureter/hydronephrosis	1/1	2/2	2/2	3/3
Umbilical artery, reversed	-/	-/	-/	1/1
<u>Cephalic Examination</u>	123/20	Not	Not	136/23
Variation	-/	Applicable	Applicable	-/

^aNumber fetuses/litters examined

^b-/ = zero

^c = red marks related to removal from the uterus during caesarian section

Chi-Square analysis, $p > 0.05$, analysis confined to differences greater than three between the control and a given treated group

Table 6. Skeletal observations and variations in fetuses from Sprague-Dawley rats administered MSM orally from gestation days 6 to 20

	Treatment Group			
	Control	Low	Mid	High
Number Examined: Fetuses/Litters	122/20	141/24	145/23	138/23
# Normal	82/20	104/24	103/23	87/23
% Normal	67.2	73.8	71.0	63.0
Skull Variations (incomplete ossification, IO)	14/9 ^a	12/8	16/10	28/12
Interparietal incomplete ossification	12/8	12/8	16/10	27/12*
1 Bone/pair of bones	12/8	12/8	16/10	23/11
2 Bones/pairs of bones	1/1	-/ ^b	-/-	2/2
3 Bones/pairs of bones	1/1	-/-	-/-	3/3
Sternebrae Variations	-/-	-/-	1/1	3/3
Centers 5&6 only	-/-	-/-	-/-	2/2
1 Center plus 5&6	-/-	-/-	1/1	1/1
Ribs Variations	21/11	24/13	26/13	28/10
Cervical rib	-/-	-/-	1/1	-/-
Rudimentary 14 th rib	21/11	20/13	24/11	23/9
Supernumerary 14 th rib	-/-	-/-	-/-	-/-
Wavy/Bulbous	1/1	4/2	1/1	5/3
Thoracic/ Lumbar Centrae Variations	7/4	4/4	8/5	4/3
1 Center dumbbell, bipartite, other	4/3	3/3	4/4	4/3
2 Centers dumbbell, bipartite, other	2/2	1/1	2/1	-/-
3 Centers dumbbell, bipartite, other	1/1	-/-	2/2	-/-
Thoracic/Lumbar Vertebrae Variations	1/1	1/1	-/-	1/1
Pelvic Girdle Variations	-/-	-/-	-/-	-/-
Sacral/Caudal Centrae/Sacral Vertebrae	-/-	-/-	-/-	-/-

^a Incidence (Fetuses/Litters)^b -/- = zero; % Normal = (# Normal ÷ Number Examined) x 100* Significantly different from control, $p \leq 0.05$, Chi-Square Analysis; analysis confined to differences of greater than 3 between the control and a given treated group

Table 7. Skeletal observation scores in fetuses from Sprague-Dawley rats administered MSM orally from gestation days 6 to 20

Bone	Study Group	Number Examined^a	Score^b						
			0	1	2	3	4	5	
Skull	Vehicle Control	122/20	108/11	12/8	1/1	1/1	-/-	-/-	
	Low	141/24	129/16	12/8	-/-	-/-	-/-	-/-	
	Mid	145/23	116/13	16/10	-/-	-/-	-/-	-/-	
	High*	138/23	110/11	23/11	2/2	3/3	-/-	-/-	
Sternebrae	Vehicle Control	122/20	122/20	-/-	-/-	-/-	-/-	-/-	
	Low	141/24	141/24	-/-	-/-	-/-	-/-	-/-	
	Mid	145/23	144/22	-/-	-/-	1/1	-/-	-/-	
	High	138/23	135/20	2/2	-/-	1/1	-/-	-/-	
Ribs	Vehicle Control	122/20	100/11	-/-	21/11	1/1	-/-	-/-	
	Low	141/24	117/11	-/-	21/13	1/1	2/2	-/-	
	Mid	145/23	119/10	-/-	24/11	1/1	1/1	-/-	
	High	138/21	110/13	-/-	23/9	5/3	-/-	-/-	
Thoracic/Lumbar Centrae	Vehicle Control	122/20	115/16	4/3	2/2	1/1	-/-	-/-	
	Low	141/24	137/20	3/3	1/1	-/-	-/-	-/-	
	Mid	145/23	137/18	4/4	2/1	2/2	-/-	-/-	
	High	138/23	134/20	4/3	-/-	-/-	-/-	-/-	
Thoracic/Lumbar Vertebrae	Vehicle Control	122/20	121/19	1/1	-/-	-/-	-/-	-/-	
	Low	141/24	140/23	1/1	-/-	-/-	-/-	-/-	
	Mid	145/23	145/23	-/-	-/-	-/-	-/-	-/-	
	High	138/23	137/22	1/1	-/-	-/-	-/-	-/-	
Pelvic Girdle	Vehicle Control	122/20	122/20	-/-	-/-	-/-	-/-	-/-	
	Low	141/24	141/24	-/-	-/-	-/-	-/-	-/-	
	Mid	145/23	145/23	-/-	-/-	-/-	-/-	-/-	
	High	138/23	138/23	-/-	-/-	-/-	-/-	-/-	
Sacral/Caudal Centrae	Vehicle Control	122/20	122/20	-/-	-/-	-/-	-/-	-/-	
	Low	141/24	141/24	-/-	-/-	-/-	-/-	-/-	
	Mid	145/23	145/23	-/-	-/-	-/-	-/-	-/-	
	High	138/23	138/23	-/-	-/-	-/-	-/-	-/-	
Sacral Vertebrae	Vehicle Control	122/20	122/20	-/-	-/-	-/-	-/-	-/-	
	Low	141/24	141/24	-/-	-/-	-/-	-/-	-/-	
	Mid	145/23	145/23	-/-	-/-	-/-	-/-	-/-	
	High	138/23	138/23	-/-	-/-	-/-	-/-	-/-	

^aIncidence (Fetal/ Litter); -/- = zero incidence; ^bSCORE KEY: 0 = No visible anomaly; 1 = Variation within normal limits; 2 = Slight variation; 3 = Moderate variation; 4 = Severe variation; 5 = Malformation

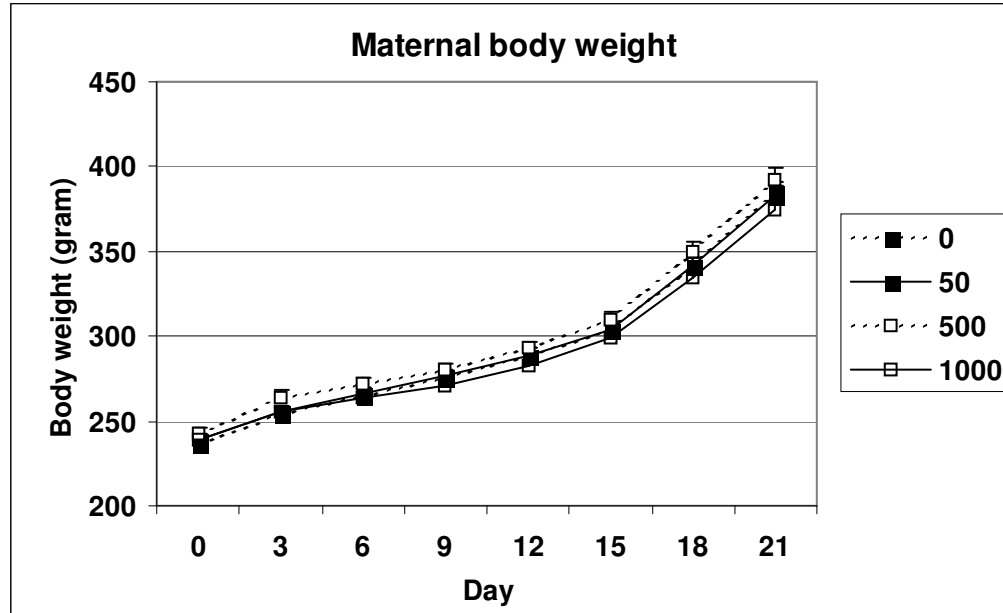


Figure 1. Body weights (mean \pm S.E.) of pregnant female Sprague-Dawley rats orally administered doses of 0, 50, 500, or 1000 mg MSM/kg/day from gestation days 6 to 20. MSM had no significant effect on body weights ($p > 0.05$). N=20 rats for 0 mg/kg group; 24 for 50 mg/kg group; and 23 for 500 and 1000 mg/kg groups