201-15159

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March 30, 2004

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Marianne Lamont Horinko, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 2216

Attn: Chemical Right-to-Know Program

Dear Administrator Horinko,

E. I. du Pont de Nemours & Company, Inc. is please to submit the proposed test plan along with the robust summary for the chemical, Monomethylformamide (MMF), CAS# 123-39-7. DuPont understands there will be a 120-day review period for the test plan and that all comments received by the EPA will be forwarded to DuPont for consideration.

This submission includes one electronic copy in .pdf format.

Please contact me by phone at (302) 773-0910 or by e-mail, Edwin.L.Mongan-1@usa.dupont.com, if you have any questions regarding this matter.

Sincerely,

Edwin L. Mongan, III
Manager, Environmental Stewardship
DuPont Safety, Health & Environment

Cc: Charles Auer – U.S. EPA
Office of Pollution Prevention & Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

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OVERALL SUMMARY FOR N-METHYL FORMAMIDE

Physical and Chemical Characteristics

N-Methylformamide (also known as monomethylformamide or MMF) is a clear, colorless liquid with a slight amine odor. MMF has a water solubility value of 1.0×10^6 mg/L, has a melting point of –3.8°C, and boils at 199.5°C. MMF has a vapor pressure of 0.253 mm Hg @ 25°C, density of 0.9961 g/cm^3 @ 25°C, a flash point of 119°C, and flammability limits of 1.8-19.7%. **Data for physical and chemical characteristics are complete and no further testing is recommended.**

Table 1: Physical and Chemical Characteristics for MMF

Melting Point	-3.8°C
Boiling Point	199.5°C
Density	0.9961 g/cm^3 @ 25°C
Vapor Pressure	0.253 mm Hg @ 25°C
Log Kow	-0.97; -1.14
Water Solubility	1.0×10^6 mg/L
Flash Point	119°C
Flammability Limits	1.8-19.7%

Environmental Fate

If released to air, a vapor pressure of 0.25 mm Hg @ 25°C indicates MMF will exist solely as a vapor in the ambient atmosphere. Vapor-phase MMF will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 57 hours. If released to soil, MMF is expected to have very high mobility based upon an estimated Koc of 0.0439. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of 2.0×10^{-8} atm-m^3/mole. MMF has been shown to biodgrade using microorganisms obtained through soil enrichment. If released into water, MMF is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. MMF, present at 400 mg/L, reached 4%, 98%, and 100% of its theoretical BOD in 3 hours, 3 days, and 7 days, respectively, using an industrial activated sludge inoculum and the Zahn-Wellens test; therefore, MMF is inherently biodegradable and expected to biodegrade in the aquatic environment. Hydrolysis is not expected since amides hydrolyze very slowly under usual environmental conditions. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low (HSDB, 2003). Consistent with behavior described above and assuming equal emissions to air, water, and soil, any residual MMF is expected to be distributed primarily to water (39.7%) and soil (59.8%) based on the Mackay Level III fugacity model. **No further environmental fate testing is recommended.**

Table 2: Environmental Fate for MMF

Bioaccumulation*	Low potential for bioaccumulation BCF = 3
Biodegradation	Readily biodegradable* 4% ThBOD in 3 hours 98% ThBOD in 3 days 100% ThBOD in 7 days
Fugacity*	Air: 0.43% Water: 39.7% Soil: 59.8% Sediment: 0.073% Based on standard emission scenario: 1000 kg/h for air, water and soil
* Modeled data.	

Ecotoxicology

Existing aquatic toxicity test data can be found in Table 3. Modeling of physical/chemical parameters (i.e., Kow) and aquatic toxicity was conducted to help provide insight into the behavior in the environment and the aquatic toxicity of MMF. Syracuse Research Corporation models for estimating physical/chemical properties were used to estimate $\log_{10}$ Kow (Meylan and Howard, 1995) for subsequent use in the ECOSAR program (Table 1). ECOSAR (Meylan and Howard, 1999) was used to estimate the aquatic toxicity of MMF to green algae, daphnids (planktonic freshwater crustaceans), and fish. ECOSAR predictions are based on actual toxicity test data for classes of compounds with similar modes of action. The existing aquatic toxicity test data and ECOSAR predictions indicate that MMF is likely to be of low concern for acute toxicity to algae, invertebrates, or fish at environmentally relevant concentrations. **No further ecotoxicity testing is recommended.**

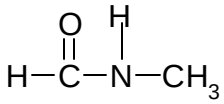
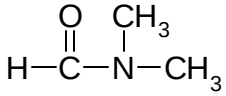
Table 1: Ecotoxicology

	MMF Test Data	**MMF Modeled Data**
Log Kow	No Data	-1.14
Toxicity to Fish (96-hour)	LC_{50} (fathead minnow) > 10,000 mg/L (N) LC_{50} (fathead minnow) > 5,000 mg/L (N)	LC_{50} = 39,170 mg/L (E)
Toxicity to Invertebrates (48-hour)	EC_{50} (*Daphnia*) > 500 mg/L (N)	EC_{50} = 33,787 mg/L (E)
Toxicity to Algae (EC_{50} value for growth inhibition)	72-hr EC_{10-90} > 8000 mg/L (N)	96-hr EC_{50} = 17,630 mg/L (E)
E = estimated value, N = value based on nominal concentrations.		

Mammalian Toxicology

While reliable MMF data are available to address most of the mammalian toxicology endpoints, certain data are lacking. The available MMF repeated dose study was 2 weeks in duration, rather than the recommended 28-day study. While numerous developmental studies were available, limited data were available regarding reproductive toxicity. With regards to genetic toxicity, an Ames test was available for MMF, but no data on clastogenicity were available. In order to strengthen the mammalian toxicologic database for MMF, supporting data for DMF (a structural analog) are provided.

Table 4: Structural Analogs

Chemical	CAS Number	Structure
N-Methylformamide (MMF)	123-39-7	
Dimethylformamide (DMF)	68-12-2	

DMF is closely structurally related to MMF (see Table 4 above), in that both contain an N-substituted formamide moiety. The substances differ only in the degree of substitution on the nitrogen atom; MMF contains one methyl group and DMF contains two. Review of the toxicologic databases for MMF and DMF indicates that the two substances have generally similar toxicity profiles. For those areas where DMF data is being used as a structural analog to provide supporting data for MMF, detailed robust summaries are provided in this document. In addition, physiochemical properties of DMF are generally similar to MMF and are provided in the detailed robust summary format.

The pathways for biotransformation of DMF and MMF have been extensively investigated. Qualitatively, the pathways of metabolism for DMF and MMF are quite similar. The major pathway for primary metabolism of DMF is the P450-mediated oxidation to form *N*-(hydroxymethyl)- *N*-methylformamide (HMMF). An alternative pathway for biotransformation of DMF is formal demethylation to yield MMF. MMF is further metabolized by hydroxylation of the remaining methyl group to form *N*-(hydroxymethyl) formamide, or by oxidation of the formyl carbon, leading to formation of a reactive carbamoylating intermediate. The reactive intermediate can react with cellular glutathione (GSH) to yield SMG, which is eventually excreted in the urine as the corresponding mercapturic acid. A more detailed discussion of the metabolism of DMF and MMF is presented at the end of the toxicity section.

Mammalian Acute Toxicity

MMF has slight acute oral toxicity with an LD_{50} in rats of 4000 – 7077 mg/kg and an LD_{50} in mice of approximately 2600 mg/kg. MMF was moderately toxic by skin absorption in the pregnant rabbit with an ALD of 1500 mg/kg and exhibited very low toxicity by skin absorption in the pregnant rat with an ALD of 11,000 mg/kg. The ALD studies were conducted in pregnant

rats and rabbits as the dose selection portion of an embryotoxicity study. MMF was irritating to rabbit eyes. No information was available on dermal irritation or sensitization. **No further acute toxicity testing is recommended for MMF.**

Table 5: Mammalian Acute Toxicity

Oral LD_{50}	4000 – 7077 mg/kg (rat) 2600 mg/kg (mice)
4-hour Inhalation ALC	> 10.76 mg/L (rat)
Dermal ALD	11,000 (rat) 1500 (rabbit)
Eye Irritation	Irritating

Repeated Dose Toxicity

The primary target organ in repeated dose studies appears to be the liver for both MMF and DMF. These effects appear at similar doses/exposures to the 2 chemicals. In a two-week inhalation study with MMF, no adverse effects were seen in rats exposed to 50 ppm. Higher concentrations (132 and/or 402 ppm) produced compound-related biochemical and microscopic pathology changes in the liver. Longer term repeated dose studies of MMF were not available; however, data were available on the structurally similar compound, DMF. In a two-week inhalation study in rats with DMF, increased liver weights were observed at 91 ppm. In a 90-day inhalation study with DMF, evidence of hepatocellular injury was seen as early as day 4 on increases in the activities of liver-specific enzymes at concentrations of 200 ppm and above. Relative liver weights were increased in males at 100 ppm and above and in females at 50 ppm and above. Pathologic changes of the liver (minimal to moderate centrilobular hepatocellular necrosis) were observed at 400 ppm and above. **No further repeated dose toxicity testing is recommended.**

Table 6: Repeated Dose Toxicity

	MMF	**DMF**
Two-Week Inhalation Study	NOEL = 50 ppm Pathologic changes in the liver and increased liver weights at 132 and/or 402 ppm.	NOEL < 91 ppm Increased liver weights were observed at 91 ppm.
90-Day Inhalation Study	No Data	NOEL< 50 ppm Biochemical changes in liver enzymes at 200 ppm and above in rats. Increased relative liver weights at 50 ppm and above in rats and mice. Pathologic changes in the liver at 400 ppm and above in rats and 50 ppm and above in mice.

Developmental Toxicity

For both MMF and DMF, the fetus appears to have the same sensitivity to the test chemicals as the maternal animal.

MMF did not produce developmental effects at maternally non-toxic doses when given by inhalation to rats (Rickard et al., 1995), dermally to rats (Stula and Krauss, 1977), and orally to rats (Kelich et al., 1995; Merkle and Zeller, 1980) and rabbits (Kelich et al., 1995). Developmental effects were observed in mice when treated with MMF orally and dermally (Roll and Baer, 1967). The quality of these studies vary, ranging from scientifically rigorous study design and thorough reporting (Kelich et al., 1995; Rickard et al., 1995), to studies with little study design and reporting details (e.g. maternal toxicity not reported or differential toxicity reported with limited study information) (see Section 5.3 for complete listing of studies). In studies in which both maternal and fetal effects were carefully examined (Kelich et al., 1995; Rickard et al., 1995), the effects of MMF appeared at the same dose levels in maternal and fetal animals. In the inhalation study in rats (Rickard et al., 1995), maternal lethality and toxicity was demonstrated at 150 ppm MMF and maternal toxicity remained evident as mild respiratory distress in the 50 ppm treated dams. Decreased fetal weight and fetal malformations and variations were observed at 150 ppm. Developmental toxicity, expressed as slight depression in fetal weight, was evident at 50 ppm. The NOEL for both the dam and the fetus was 15 ppm. In an oral study in rats and rabbits (Kelich et al., 1995), maternal toxicity was evidenced as decreased body weight and food consumption at 75 mg/kg in rats and 50 mg/kg in rabbits. Developmental toxicity was evidenced by reduced fetal viability, reduced fetal weight, and fetal malformations at 75 mg/kg in rats and 50 mg/kg in rabbits. The NOEL for maternal and fetal toxicity was 10 mg/kg in both rats and rabbits.

Extensive testing has been conducted in rats and rabbits by inhalation, oral, and dermal routes of exposure which shows that DMF affects the embryo/fetus only under conditions which will affect the maternal animal. Rats exposed by inhalation to either 18 or 172 ppm during gestation showed no structural changes. Both maternal and fetal weights were reduced at 172 ppm (Kimmerle and Machener, 1975). Similarly, no teratogenic effects were seen in rats inhaling either 30 or 300 ppm during gestation with weights affected in both maternal and fetal rats at 300 ppm (Lewis et al., 1992). Oral studies in rats showed maternal toxicity at doses of 100 mg/kg or greater along with fetal toxicity at the same doses. No malformations were seen and the fetal effect consisted of weight depressions and skeletal developmental delays (Saillenfait et al., 1997). DMF given orally to rabbits produced both maternal and fetal effects with fetal anomalies being produced at doses that had little maternal effect (Merkle and Zeller, 1980). Studies in other species and those involving dermal exposure supports the hypothesis that the maternal and fetal animals are equally sensitive to the toxic effects of DMF (Kennedy, 1986; 2001). **No further developmental toxicity testing is recommended.**

Table 7: Developmental Toxicity

	MMF	**DMF**
Inhalation Study in rats	Maternal and fetal NOEL = 15 ppm	Maternal and fetal NOEL = 18 ppm Maternal and fetal NOEL = 30 ppm
Oral Study in rats	Maternal and fetal NOEL = 10 mg/kg	Maternal and fetal NOEL = 50 mg/kg
Oral Study in rabbits	Maternal and fetal NOEL = 10 mg/kg	Maternal NOEL = 65 mg/kg Fetal NOEL = 44.1 mg/kg

Reproductive Toxicity

No formal reproductive toxicity studies have been conducted on MMF. Data were available on the structurally similar compound, DMF. Rats given dermal applications of either 500, 1000, or 2000 mg/kg of DMF from 4 weeks pre-mating through the production and lactation of 1 litter showed no effects on reproductive endpoints. Administration of 2000 mg/kg resulted in a reduction in the number of viable pups per litter. Body weight effects were seen in the parents at 1000 and 2000 mg/kg. Fetal and weanling body weights were similar to controls. In a continuous breeding study in which mice were exposed to either 1000, 4000, or 7000 ppm of DMF in their drinking water, a decrease in fertility (reflected by a decrease in pups born alive and in live litter size) was seen at 4000 and 7000 ppm. Liver damage was produced in all parental animals (1000 to 7000 ppm) and body weight gains were affected at 4000 and 7000 ppm. Decreased fertility and fetal effects (decreased pup weight) paralleled the parental sensitivity observed at 4000 and 7000 ppm. No effects on fertility or fetal parameters were observed at 1000 ppm. In a 90-day inhalation study conducted in rats and mice, relative testes weights were increased at 400 ppm and above in the rats; however, no microscopic findings or adverse effects on sperm density or motility were observed in rats or mice. DMF is not considered a unique reproductive toxicant (any reproductive effects have been shown to occur at higher doses/exposures than hepatotoxic effects). Based on its structural similarity and its

similar toxicity profile for developmental and repeated dose toxicity, we expect MMF to not be a reproductive toxin. **No reproductive testing is recommended for MMF.**

Table 8: Reproductive Toxicity

	MMF	**DMF**
Reproductive Toxicity	No Data	Not a unique reproductive toxin* in a 1-generation study or a continuous breeding study
* = reproductive effects have been shown to occur at higher doses/exposures than hepatotoxic effects		

Genetic Toxicity

MMF was not mutagenic in *Escherichia coli*. No data on the clastogenicity of MMF are available. However the genetic toxicity of the analog, DMF, has been extensively investigated. A review of the available literature indicates that although some positive findings have been observed, DMF does not induce chromosome aberrations or gene mutations in most of the systems tested. In *in vitro* bacterial mutation assays, 33/37 tests with DMF produced negative results. DMF was also negative in 14/14 unscheduled DNA synthesis assays (*in vitro*), negative in 19/22 clastogenicity assays (*in vitro*), negative in 8/9 *in vivo* micronucleus assays, negative in 11/11 *in vivo* dominant lethal tests, and negative in 17/17 other *in vivo* genetic toxicity assays. The weight of evidence suggests that DMF and, by analogy, MMF are not genotoxic. **No further genotoxicity testing is recommended for MMF.**

Table 9: GeneticToxicity

	MMF	**DMF**
Mutagenicity	Not mutagenic	Not mutagenic
Clastogenicity	No Data	Not clastogenic

Metabolism

The pathways for biotransformation of N,N-dimethylformamide (DMF) and N-methylformamide (MMF) have been the subject of extensive investigation for over 30 years. The primary driver behind this effort has been the realization that the toxicity of these compounds is intrinsically related to their metabolism, both quantitatively and qualitatively. The primary pathways for biotransformation of DMF and MMF are illustrated in Figure 1. The major pathway for primary metabolism of DMF in all species studies, including humans, is the P450-mediated oxidation of one of the *N*-methyl moieties to form the stable carbinolamide *N*-(hydroxymethyl)-*N*-methylformamide (HMMF, pathway 1) (Gescher, 1993; Van den Bulcke et al., 1994; Hundley et al., 1993a; 1993b; Lareo and Perbelline, 1995). Several lines of evidence indicate that CYP2E1 is the major catalyst for this reaction (Mráz et al., 1993). HMMF is readily excreted in the urine, accounting for approximately 50% of the administered dose of DMF in rats and 22% of the dose in human volunteers exposed by inhalation (Van den Bulcke et al., 1994; Mráz and Nohova, 1992). An alternative pathway for biotransformation of DMF involves formal demethylation to

yield MMF (pathway 2). Demethylation of DMF to MMF is thought to occur both directly (Scailteur and Lauwerys, 1984) and via hydrolysis of HMMF (pathway 2a) (Van den Bulcke et al., 1994).

MMF thus formed is further metabolized by two distinct pathways. The first pathway involves hydroxylation of the remaining methyl group to form *N*-(hydroxymethyl)formamide (HMF, pathway 4), analogous to the oxidation of DMF to HMMF (Kestell et al., 1985; Tupil and Timbrell, 1988). Urinary HMF accounted for approximately 3-6% and 7-9% of the administered dose of MMF in rats and mice, respectively. HMF has not been quantified following administration of DMF, but approximately 13% of the administered dose of DMF in a study involving human volunteers was recovered in the urine as formamide, most of which was thought to result from thermal decomposition of HMF in the analytical system (Mráz and Nohova, 1992). By analogy with HMMF, a small amount of MMF is thought to undergo demethylation to formamide, presumable involving the intermediacy of HMF (pathway 5) (Kestell et al., 1985). The second pathway for further metabolism of MMF is oxidation of the formyl carbon (pathway 3), leading to formation of a highly reactive intermediate thought to play a pivotal role in toxicity of both DMF and MMF. This reaction appears to be catalyzed primarily, if not exclusively, by CYP2E1 (Mráz et al., 1993; Chieli et al., 1995). The existence of this intermediate has been inferred from the detection of *S*-(*N*-methylcarbamoyl)glutathione (SMG) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMMC). The metabolite generated by oxidation of the formyl moiety has not been unequivocally identified, but methyisocyanate has been proposed as a likely candidate, as shown in figure 1 (Gescher, 1993). However, other intermediates such as *N*-methylcarbamic acid have not been ruled out (Gescher, 1993; Kestell et al., 1985). There is some evidence to suggest that HMMF may also be oxidized at the formyl carbon, generating the reactive intermediate following hydrolysis of the *N*-hydroxymethyl group (pathway 3), though this route can at most account for ~5% of the total carbamoylating intermediate (Van den Bulcke et al., 1994; Mráz et al., 1993). As indicated in Figure 1, the reactive intermediate can participate in covalent binding to proteins and transcarbamoylation reactions, and is thought to be directly responsible for hepatotoxicity of both DMF and MMF. The carbamoylating intermediate can react with cellular glutathione (GSH) to yield SMG (pathway 6), which has been detected as a biliary metabolite of DMF and MMF (Gescher, 1993). SMG proceeds through the mercapturic acid pathway (pathway 7), and is eventually excreted in the urine as AMMC. Quantitation of urinary AMMC has been proposed as a useful biomarker for occupational exposure to DMF (Lareo and Perbellini, 1995; Sakai et al., 1995). Human volunteers exposed to 30 mg/m^3 DMF excreted approximately 13% of the dose as AMMC (Mráz and Nohova, 1992). As indicated previously, the formation of SMG is reversible, and this metabolite can be hydrolyzed to form methylamine, as could AMMC. Alternatively, methylamine could be formed by direct hydrolysis, or decomposition of the carbamoylating intermediate (pathway 8). The involvement of the reactive intermediate in methylamine formation has been inferred from the prominent kinetic deuterium isotope effect on methylamine formation observed following carbamoyl-^{2}H-MMF in mice (Threadgill et al., 1987).

Pharmacokinetic studies of DMF in various species have demonstrated that the area under the plasma concentration vs. time curves (AUC) for DMF increases in the order monkey < mouse < rat following inhalation exposure to comparable concentrations (Hundley et al., 1993a; 1993b). Further, the AUC was found to increase out of proportion with increased exposure concentration,

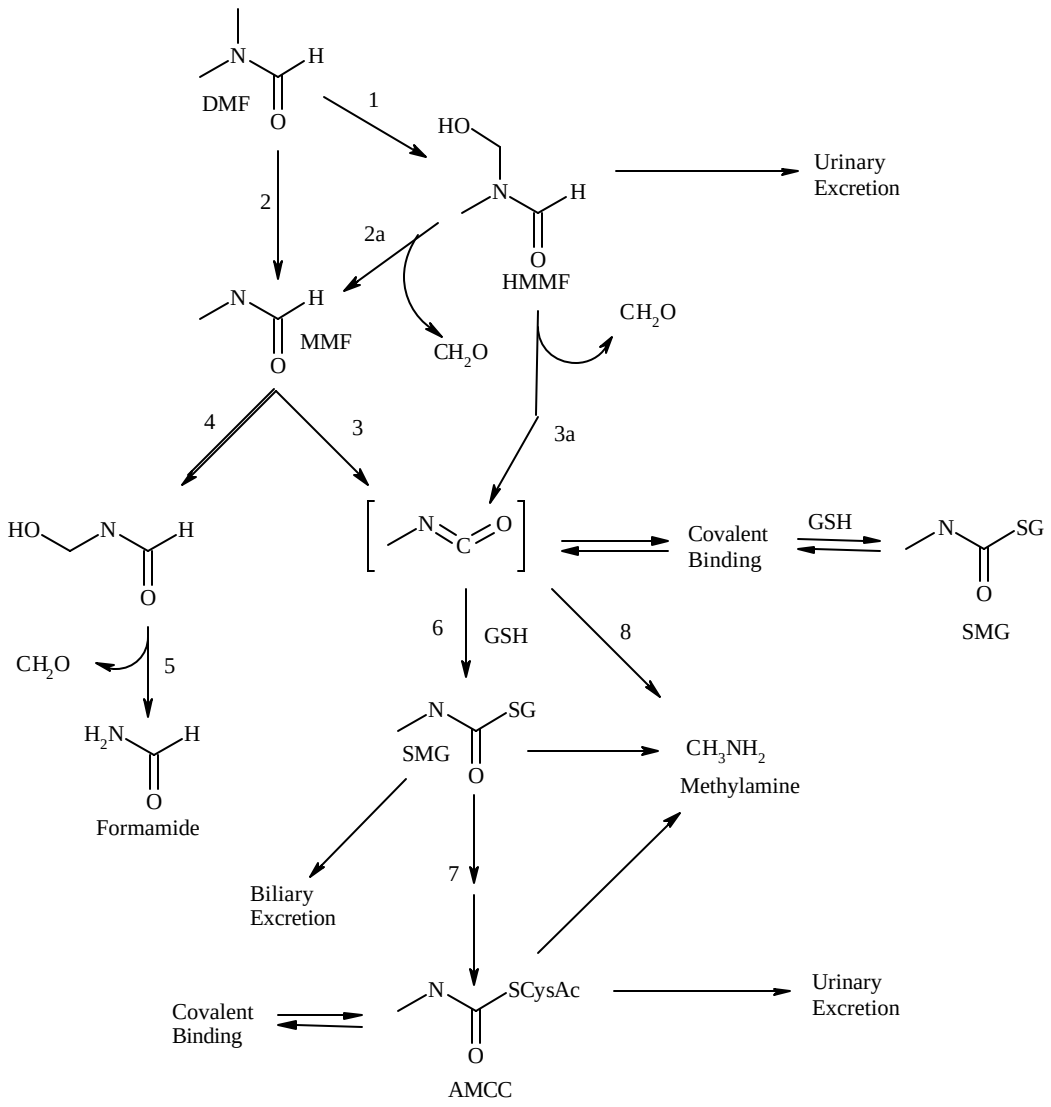
suggesting saturation of DMF metabolism in all thee species. In all species, the major urinary metabolite was HMMF, followed by DMF and MMF (Hundley et al., 1993a; 1993b). AMMC was not measured in these studies. Comparative excretion studies of DMF in rodents and humans have demonstrated significant quantitative differences in urinary metabolite profiles following i.p. (rodents) or inhalation (humans) exposure (Mráz et al., 1989). In this study, only minute concentration of DMF were detected. Differences in the proportion of the dose excreted unchanged between this and other (Hundley et al., 1993a) studies may be due in part to differences in the route of administration. Excretion of HMMF was greatest in rats (36.8%, followed by humans (25.9%) and mice (8.4%). Formamide (representing formamide + HMF) accounted for 23-38% of the dose in rodents and approximately 14% of the dose in humans. Approximately 1.6-5.2% of the dose was excreted at AMMC in rodents, while humans excreted an average of 14.2% of the dose as AMMC. Following exposure to DMF, excretion of HMMF and MMF are rapid, while excretion of AMMC is delayed, particularly in humans (Mráz and Nohova, 1992). In rodents, hepatotoxicity of DMF is delayed at higher doses compared to lower doses, and this is though to result from the inhibition of metabolism of MMF to reactive species by DMF. Consistent with this idea is the finding that the K_M value for metabolism of MMF to SMG is approximately 20 fold higher than the K_M for metabolism of DMF to HMMF (Mráz et al., 1993). Further, both the metabolism and the hepatotoxicity of MMF were delayed in rats treated simultaneously with DMF (Van den Bulcke et al., 1994; Lundberg et al., 1983). In addition, DMF induces its own metabolism in mice and rats, with lower plasma AUCs observed following repeated exposure compared to single exposure (Hundley et al., 1993a). This effect was not observed in monkeys exposed repeatedly to DMF by inhalation (Hundley et al., 1993b). However, there was a shift in the balance of urinary metabolites in male monkeys, suggesting slightly greater metabolism of DMF to HNNF and MMF following repeated exposure.

In mice, the plasma half-life of MMF administered by *i.p.* injection was approximately 3.6 hours (Gescher et al., 1982). When MMF was administered to rats and mice, a significant fraction of the administered dose was excreted as unchanged MMF in the urine (Tupil and Timbrell, 1988). Excretion of unchanged MMF was greater in rats (23-40%) than in mice (10-12%). In mice, methylamine was the major urinary metabolite (~30% of the dose), whereas this was a comparatively minor metabolite in rats (<10%). Approximately 3-6% of the administered dose was excreted as AMMC in both species. In mice, greater than 90% of the AMMC was excreted within the first 24 hours after dosing with MMF. However, in rats, only about 35% of the total AMMC was excreted in the first 24 hours, with the remainder excreted between 24 and 48 hours. These data are consistent with the generally more rapid metabolism of MMF and greater severity of hepatotoxicity in mice compared to rats, and suggest that the rat may be a more appropriate model for MMF toxicity than the mouse.

Overall, the pathways of metabolism for DMF and MMF are qualitatively similar. Hepatotoxicity of both compounds is thought to be mediated by a reactive carbamoylating intermediate formed by oxidation of the formyl carbon of MMF. Consequently, the hepatotoxicity of the two compounds is qualitatively similar. However, due to inhibition of formyl oxidation step by DMF itself, the hepatotoxicity of the latter compound is delayed with respect to time of exposure.

(March 30, 2004)

Figure 1. Metabolic Pathways for Dimethylformamide (DMF) and N-Methylformamide (MMF)



Human Exposure

The predominant use (greater than 95%) of monomethylformamide (MMF) is as a DuPont-limited intermediate. Less than 5% of DuPont's production is sold to external customers. These customers, located in Europe and Japan, use MMF for industrial purposes only: as a solvent in electronics manufacture and as a solvent for chemical synthesis of resins. Personal protective equipment and ventilation are used at these sites to minimize worker exposure.

For the major use, MMF is manufactured at one DuPont plant and is shipped by railcar to another DuPont facility for use as a raw material. MMF is catalytically converted *in-situ* to

another substance, which is then further reacted to form downstream products at the site. The manufacture and use are closed system operations. The only potential human exposure occurs during loading and unloading.

Monomethylformamide is made on a campaign basis twice a year. Monomethylformamide is produced in a closed system and is hard piped to loading spots. Flex hose is connected to the liquid valve on the tank car and the liquid is fed through an induction pipe to the bottom of car. The dome lid is kept down except during periods when operators are performing level checks and collecting samples. Off-gases associated with the MMF process are vented to a flare stack. MMF is shipped by rail to the DuPont use site and is unloaded into a storage tank and is consumed throughout the year. At the DuPont use site, MMF is handled in a closed system except when piping connections are made during the unloading process. A nitrogen pad is kept on the railcar during the unloading process and any vented material goes directly to an incinerator. All transfers from the storage tank and subsequent consumption in the reactor occur under closed system conditions, without exposure to workers.

At the DuPont manufacturing site, potential for employee exposure is greatest when operators perform periodic checks on top of MMF railcars to inspect the dome, do level checks, and collect samples. Operators wear appropriate personal protective equipment (PPE) to protect themselves from liquid and vapor contact while on the railcar. PPE consists of a positive pressure air supplied respirator, Nomex coveralls, and neoprene gloves. Safety showers, eyewash stations, and self-contained breathing apparatus (SCBA) are available in close proximity to the operations area. Chemical splash goggles, Nomex coveralls and neoprene gloves are required by all personnel for patrol-type work during the production of MMF.

At the DuPont use site, employee potential for exposure occurs only during unloading operations. When making connections to lines containing MMF, chemical suit, boots, NIOSH approved hood respirator, and gloves are worn. Chemical gloves, chemical jacket, chemical splash goggles, and faceshield are required for sampling activities involving MMF.

The DuPont Acceptable Exposure Limit (AEL) for monomethylformamide is 2 ppm as an 8- and 12-hour TWA (time-weighted average). Air monitoring has been conducted on monomethylformamide and all measured concentrations are well below the AEL. Results are shown in the table below:

(March 30, 2004)

Exposure Data:

Job Sampled	No. of Results	Average (ppm)	Minimum (ppm)	Maximum (ppm)
DuPont Manufacturing Site Operators (full shift)	**18**	**<0.1**	**<0.1**	**<0.1**
DuPont Manufacturing Site Loaders	**18**	**0.162**	**<0.1**	**0.6**
DuPont Use Site Unloaders (full shift)	**13**		**All <0.1**	

Conclusion

Adequate data are available to address all the required endpoints. A substantial body of data exists for MMF *per se*. Where data are lacking on MMF, reliable data are available for the close analog, DMF. The use of DMF data to supplement the existing mammalian toxicity data for MMF is supported by the close similarity in molecular structure, similarity in physical/chemical properties, and the similarity in toxicity observed where data for both substances are available for comparison. Further strong support for use of DMF as an analog for MMF is provided by the extensive understanding of the metabolic fate of DMF and MMF, and the fact that MMF is one of the products of metabolism of DMF. The use of DMF as an analog to MMF is consistent with the Agency's directive to HPV participants to maximize the use of scientifically appropriate data for related chemicals. Although some differences between MMF and DMF may be expected, we believe these differences to be minimal and insufficient to warrant additional animal testing.

TEST PLAN FOR N-METHYL FORMAMIDE

N-Methyl formamide **CAS No. 123-39-7**	**Data Available**	**Data Acceptable**	**Testing Required**
Study	**Y/N**	**Y/N**	**Y/N**
PHYSICAL/CHEMICAL CHARACTERISTICS			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
ENVIRONMENTAL FATE			
Photodegradation	Y	Y	N
Stability in Water	Y	Y	N
Transport (Fugacity)	Y	Y	N
Biodegradation	Y	Y	N
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	N
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y*	Y	N
Developmental Toxicity	Y	Y	N
Reproductive Toxicity	Y*	Y	N
Genetic Toxicity Gene Mutations	Y*	Y	N
Genetic Toxicity Chromosomal Aberrations	Y*	Y	N
Y = yes; N= no; NR = not required * = Data are available on an analog chemical.			

References for Summary

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Lareo, A. C. and L. Perbellini (1995). Int. Arch. Occup. Environ. Health, 67:47-52.

Lewis, S. C. et al. (1992). Drug. Chem. Toxicol., 15(1):1-14.

Lundberg, I. et al. (1983). Toxicol. Lett., 17:29-34.

Merkle, J. and H. Zeller (1980). Arzneimittel-Forsch. (Drug Res.), 30(9):1557-1562 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

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Mráz, J. and H. Nohova (1992). Int. Arch. Occup. Environ. Health, 64:85-92.

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Scailteur, V. and R. Lauwerys (1984). Chem.-Biol. Interact., 50:327-337.

Stula, E. F. and W. C. Krauss (1977). Toxicol. Appl. Pharmacol., 41:35-55.

Threadgill, M. D. et al. (1987). J. Pharmacol. Exp. Ther., 242:312-319.

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ROBUST SUMMARY FOR N-METHYL FORMAMIDE

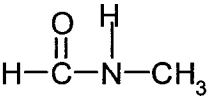
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 123-39-7

Chemical Name: N-methyl formamide

Structural Formula:



Other Names:
EK 7011
Formamide, N-methyl-
Formic acid amide, N-methyl-
Methylformamide
Monomethylformamide (MMF)
N-Methylformamide (NMF)
N-Methylformic acid amide
N-Methylformimidic acid
N-Monomethylformamide
NCS 3051
X 188

Exposure Limits: 2 ppm (8- and 12-hour TWA), skin DuPont AEL

RECEIVED OPPT CBIC 04 APR -2 AM 10: 03

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-3.8°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Lide, D. R. (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC Press Inc., New York.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point:

DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).

Budavari, S. (ed.). (1996). The Merck Index -An Encyclopedia of Chemicals, Drugs, and Biologicals, p. 1037, Merck and Co., Inc., Whitehouse Station, NJ (HSDB/100).

Riddick, J. A. and W. B. Bunger (1970). Techniques of Chemistry, Vol. II. Organic Solvents, Physical Properties and Methods of Purification, Wiley-Interscience, New York, NY (NISC/IS-0006670).

Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	-61°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Bipp, H. and H. Kieczka (1989). In Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Vol. A12, pp. 1-12, VCH Verlagsgesellschaft, Weinheim (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Not assignable because limited study information was available.

2.2 Boiling Point

Value:	199.5ºC
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Lide, D. R. (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC Press Inc., New York.
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point:

DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).

Budavari, S. (ed.). (1996). The Merck Index -An Encyclopedia of Chemicals, Drugs, and Biologicals, p. 1037, Merck and Co., Inc., Whitehouse Station, NJ (HSDB/100).

Grasselli, J. G. and W. M. Ritchey (1975). Chemical Rubber Company Atlas of Spectral Data and Physical Constants for Organic Compounds, 2nd ed., CRC Press, Inc., Cleveland, Ohio (NISC/IS-0006671).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 10th ed., p. 3678, John Wiley & Sons, Inc., New York.

Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	152.5-153.5°C
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Bipp, H. and H. Kieczka (1989). In Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Vol. A12, pp. 1-12, VCH Verlagsgesellschaft, Weinheim (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Not assignable because limited study information was available.

2.3 Density

Value:	0.9961 g/cm^3
Temperature:	25°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Budavari, S. (ed.). (1996). The Merck Index -An Encyclopedia of Chemicals, Drugs, and Biologicals, p. 1037, Merck and Co., Inc., Whitehouse Station, NJ (HSDB/100).
Reliability:	Not assignable because limited study information was available.

Additional References for Density:

DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).

Lide, D. R. (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC

Press Inc., New York.

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 10th ed., p. 3678, John Wiley & Sons, Inc., New York.

Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	0.95 g/cm^3
Temperature:	25°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Bipp, H. and H. Kieczka (1989). In Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Vol. A12, pp. 1-12, VCH Verlagsgesellschaft, Weinheim (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Not assignable because limited study information was available.

2.4 Vapor Pressure

Value:	0.253 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis,Washington, DC (HSDB/100).
Reliability:	Not assignable because limited study information was available.

Additional References for Vapor Pressure:

DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).

Riddick, J. A. and W. B. Bunger (1970). Techniques of Chemistry, Vol. II. Organic Solvents, Physical Properties and Methods of Purification, Wiley-Interscience, New York, NY (NISC/IS-0006672).

Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	3.6 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	IPCS (International Programme on Chemical Safety) (1991). Environmental Health Criteria 114 "Dimethylformamide" United Nations Environment Programme, International Labour Organisation, World Health Organization; 1-124 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Not assignable because limited study information was available.

2.5 Partition Coefficient (log Kow)

Value:	-0.97
Temperature:	No Data
Method:	No Data
GLP:	Not Applicable
Reference:	Hansch, C. et al. (1995). Exploring QSAR - Hydrophobic, Electronic, and Steric Constants, p. 4, American Chemical Society, Washington, DC (HSDB/100).
Reliability:	Not assignable because limited study information was available.

Value:	-1.14
Temperature:	25°C
Method:	Modeled. KOWWIN, v. 1.67, module of EPIWIN 3.11 (Syracuse Research Corporation). KOWWIN uses "fragment constant" methodologies to predict log P. In a "fragment constant" method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate.
GLP:	Not Applicable
Reference:	Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional Reference for Partition Coefficient (log Kow):

Leo, A. J. (1978). Report on the Calculation of Octanol/Water Log P Values for

Structures in EPA Files (NISC/IS-0006674).

Supporting Data: DMF

Value:	-0.85
Temperature:	25ºC
Method:	Mean value of 3 measurements
GLP:	No Data
Reference:	BASF AF (1987). Unpublished data, J-No. 124659/08 (November 27) (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Valid with restrictions (reliability given in the SIDS Dossier).

2.6 Water Solubility

Value:	1.0×10^6 mg/L
Temperature:	25ºC
pH/pKa:	No Data
Method:	No Data
GLP:	Not Applicable
Reference:	Howard, P. H. and W. M. Meylan (1997). Handbook of Physical Properties of Organic Chemicals, p. 245, CRC Lewis Publishers, Boca Raton, FL (HSDB/100).
Reliability:	Not assignable because limited study information was available.

Additional References for Water Solubility:

DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).

Riddick, J. A. et al. (1985). Techniques of Chemistry, 4th ed., Volume II. Organic Solvents, p. 655, John Wiley and Sons, New York, NY (HSDB/100).

Weast, R. C. (1969). Chemical Rubber Company Handbook of Chemistry and Physics, 50th ed., CRC Press, Inc., Cleveland, Ohio (NISC/IS-0006673).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 10th ed., p. 3678, John Wiley & Sons, Inc., New York.

Lide, D. R. (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC Press Inc., New York.

Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	200 g/L
Temperature:	20°C
pH/pKa:	No Data
Method:	No Data
GLP:	No Data
Reference:	BASF AG (2000). Safety Data Sheet Dimethylformamide (January 28) (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Not assignable because limited study information was available.

2.7 Flash Point

Value:	119ºC
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).
Reliability:	Not assignable because limited study information was available.

Additional References for Flash Point:

Snyder, R. (ed.) (1990). Ethyl Browning's Toxicity and Metabolism of Industrial Solvents, 2nd ed. Volume II: Nitrogen and Phosphorus Solvents, p. 169, Elsevier, Amsterdam-New York-Oxford (HSDB/100).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 10th ed., p. 3678, John Wiley & Sons, Inc., New York.

Supporting Data: DMF

Value:	57.5ºC
Method:	Closed Cup, DIN 51 755
GLP:	Unknown
Reference:	BASF AG (1979). Unpublished data, TKM/SIK 79/0518 (May 22) (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Valid with restrictions (reliability given in the SIDS Dossier)

2.8 Flammability

Results:	Autoignition: 323ºC Explosive limits: 1.8 - 19.7%

Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. B0000030 (February 2).
Reliability:	Not assignable because limited study information was available.

Additional References for Flammability: None Found.

Supporting Data: DMF

Results:	Autoignition: 410ºC
Method:	DIN 51 794
GLP:	Unknown
Reference:	BASF AG (1979). Unpublished data, TKM/SIK 79/0518 (May 22) (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).
Reliability:	Valid without restriction (reliability given in the SIDS Dossier)

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	Not expected due to lack of adsorption >290 nm
Indirect Photolysis:	57 hour half-life due to OH radical reactions
Breakdown Products:	No Data
Method:	Modeling and inspection of adsorption spectrum
GLP:	Not Applicable
Reference:	Direct Photolysis: Judith C. Harris (1990). Rate of Aqueous Photolysis. Ch. 8 In W. J. Lyman, W. F. Reehl, & D. H. Rosenblatt (ed.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC. Indirect Photolysis: AOPWIN, v. 1.91 module of EPIWIN 3.11. Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-99.
Reliability:	Estimated value based on accepted model.

Additional Reference for Photodegradation:

Data from this additional source were not summarized because insufficient study information was available.

Mudder, T. I. (1982). Am. Chem. Soc., Div. Environ. Chem. Cong., Kansas City, pp. 52-53 (cited in Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York).

3.2 Stability in Water

Concentration:	No Data
Half-life:	N-Methylformamide is not expected to undergo hydrolysis in the environment since amides hydrolyze very slowly under environmental conditions (Mabey and Mill, 1978)
% Hydrolyzed:	No Data
Method:	No Data
GLP:	Not Applicable
Reference:	Mabey, W. and T. Mill (1978). J. Phys. Chem. Ref. Data., 7:383-415 (HSDB/100).
Reliability:	Estimated value based on accepted model.

Concentration:	No Data
Half-life:	Hydrolysis Rate Extremely Slow or t1/2 > 1 Year
% Hydrolyzed:	No Data
Method:	Modeled. HYDROWIN, v. 1.67 module of EPIWIN v3.11 (Syracuse Research Corporation). HYDROWIN estimates aqueous hydrolysis rate constants for the following chemical classes: esters, carbamates, epoxides, halomethanes and selected alkyl halides. HYDROWIN estimates acid- and base-catalyzed rate constants; it does NOT estimate neutral hydrolysis rate constants. The prediction methodology was developed for the U.S. Environmental Protection Agency and is outlined in Mill et al., 1987. Half-life prediction is based on the observation of an amide group on SMILES atom 2, not on a QSAR equation. "Compound has an amide group; C=O located at SMILES atom #: 2"
GLP:	Not Applicable
Reference:	Mill, T. et al. (1987). "Environmental Fate and Exposure Studies Development of a PC-SAR for Hydrolysis: Esters, Alkyl Halides and Epoxides" EPA Contract No. 68-02-425, SRI International Menlo Park, CA. Harris, J. C. (1990). Rate of Hydrolysis. Ch. 7 In W. J. Lyman, W. F. Reehl, & D. H. Rosenblatt (ed.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity):

Media: Air, Water, Soil, and Sediments

Level III Inputs:

SMILES Code: O=CNC
Molecular Wt: 59.07
Henry's LC: 1.97e-008 atm-m^3/mole (Henry database)
Vapor Press : 0.253 mm Hg (user-entered)
Log Kow: -0.97 (Kowwin program)
Soil Koc: 0.0439 (calc by model)

Distributions:

Compartment	% of total distribution*	½ life (advection + reaction)
Air	0.43	57
Water	39.7	360
Soil	59.8	720
Sediment	0.073	3240

* - based on standard emission scenario:1000 kg/h each for air, water and soil

Adsorption Coefficient: Koc = 0.0439

Desorption: No Data

Volatility: Henry's Law Constant = 1.97e-008 atm-m^3/mole

Method: Modeled.

Henry's Law Constant - HENRYWIN v. 3.10 module of EPIWIN v3.11 (Syracuse Research Corporation). Henry's Law Constant (HLC) is estimated by two separate methods that yield two separate estimates. The first method is the bond contribution method and the second is the group contribution method. The bond contribution method is able to estimate many more types of structures; however, the group method estimate is usually preferred (but not always) when all fragment values are available.

Koc – Calculated from Kow by the Mackay Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation).

Environmental Distribution - Mackay Level III fugacity model, in EPIWIN v3.11 (Syracuse Research Corporation).

	Emissions (1000 kg/hr) to air, water, and soil compartments.
GLP:	Not Applicable
Reference:	HENRYWIN –

J. Hine and P. K. Mookerjee (1975). J. Org. Chem., 40(3):292-298.

Meylan, W. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-1293.

Fugacity - The methodology and programming for the Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation) were developed by Dr. Donald MacKay and coworkers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated values based on accepted models.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Values:

Linear Model Prediction:	0.86	Degrades Fast
Non-Linear Model Prediction:	0.99	Degrades Fast
Ultimate Biodegradation Timeframe:	3.09	Weeks
Primary Biodegradation Timeframe:	3.91	Days
MITI Linear Model Prediction:	0.76	Readily Degradable
MITI Non-Linear		

Model Prediction:	0.87 Readily Degradable
Breakdown Products:	No Data
Method:	Modeled. BIOWIN, v. 4.01 module of EPINWIN v3.11 (Syracuse Research Corporation). BIOWIN estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental microorganisms. Estimates are based upon fragment constants that were developed using multiple linear and non-linear regression analyses.
Reference:	Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-65. Howard, P. H. et al. (1992). Environ. Toxicol. Chem., 11:593-603. Howard, P. H. et al. (1987). Environ. Toxicol. Chem., 6:1-10. Tunkel, J. et al. (2000). Predicting Ready Biodegradability in the MITI Test. Environ. Toxicol. Chem., accepted for publication.
Reliability:	Estimated value based on accepted model.

Value:	4% ThBOD in 3 hours 98% ThBOD in 3 days 100% ThBOD in 7 days
Breakdown Products:	No Data
Method:	Zahn-Wellens Inherent Biodegradability test 400 mg/L initial test compound concentration An industrial activated sludge inoculum was used.
GLP:	Unknown
Reference:	BASF AG (1982). Labor Oekologie, unpublished research (cited in Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., V. 2: p. 1495, John Wiley and Sons, NY, NY.
Reliability:	Not assignable because limited study information was available.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	BCF = 3
Method:	Modeled. BCFWIN v. 2.15 module of EPINWIN v3.11

(Syracuse Research Corporation). BCFWIN estimates the bioconcentration factor (BCF) of an organic compound using the compound's log octanol-water partition coefficient (Kow) with correction factors based on molecular fragments.

GLP: Not Applicable

Reference: "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient", SRC TR-97-006 (2nd Update), July 22, 1997; prepared for: Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by: William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup and Sybil Gouchie; Syracuse Research Corp.

Reliability: Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type: **96-Hour LC_{50}**

Species: Fathead minnow, *Pimephales promelas*

Value: > 10,000 mg/L

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The test material, in original form, was introduced into commercial, glass, rectangular 21-L aquaria and diluted with laboratory well water to yield the desired exposure concentrations in 15 L final volumes. An identical vessel containing only laboratory well water was designated as the control.

Ten fathead minnows, with a 2.4 cm mean standard length and 0.15 g mean wet weight, were randomly assigned to each test vessel. The fish were not fed for 48 hours prior to nor during the exposure. The nominal test solutions (0, 1, 100, 1000, 10,000 mg/L) were not aerated. Temperature was maintained at 22ºC. Photoperiod was maintained at 16 hours light: 8 hours dark.

Mortality counts and observations were made every 24 hours during the 96-hour exposure period.

Dissolved oxygen and pH were measured in the control and 1, 1000, and 10,000 mg/L test concentrations at the

	beginning of the test, at 48-hours, and at 96-hours. Total alkalinity, hardness (EDTA), and conductivity were measured at the beginning of the test in the water control.
GLP:	No
Test Substance:	N-methylformamide, purity 97.8 mole %
Results:	No mortality was observed during the test; therefore, an LC_{50} value could not be determined, but is > 10,000 mg/L. The chemical and physical parameters measured during the test were all within acceptable limits. Dissolved oxygen ranged from 7.6-8.8, 7.8-8.8, 7.3-8.8, 7.1-8.8 in the 0, 1, 1000, and 10,000 mg/L groups, respectively. pH ranged from 7.0-7.5, 6.9-7.5, 6.8-7.4, and 6.5-6.9 for the 0, 1, 1000, and 10,000 mg/L groups, respectively. Total alkalinity, EDTA hardness, and conductivity at 0 hours in the control group were 83 mg/L as $CaCO_3$, 76 mg/L as $CaCO_3$, and 179 µmhos/cm, respectively.
Reference:	DuPont Co. (1985). Unpublished Data, Haskell Laboratory Report No. 120-85, "96-Hour LC_{50} to Fathead Minnows" (March 18).
Reliability:	Medium because a suboptimal study design with nominal concentrations was used for testing.
Type:	**96-Hour LC_{50}**
Species:	Fathead minnow, *Pimephales promelas*
Value:	> 5000 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Nominal concentrations of 0, 0.50, 1.0, 50, 500, and 5000 mg/L were used for testing. The test was unaerated and static. Dissolved oxygen and pH were measured.
GLP:	No
Test Substance:	N-methylformamide, purity 99.9%
Results:	Based on visual observation, the test substance was soluble in well water at all concentrations. No mortality was observed in any test concentration. All fish in the highest test concentration (5000 mg/L) were noted to be breathing rapidly after 24 hours of exposure and for the duration of the test.

	Loading (0.15 g/L at test end) was within acceptable limits. Dissolved oxygen concentrations were 8.3, 8.3, 8.3, 8.3, 8.3, 8.3 mg/L at 0 hours and 4.5, 5.6, 5.4, 5.5, 5.1, and 2.2 mg/L at 96 hours in the 0, 0.50, 1.0, 50, 500, and 5000 mg/L groups, respectively. pH values were 7.6, 7.6, 7.6, 7.6, 7.5, and 7.2 at 0 hours and 7.3, 7.3, 7.3, 7.2, 7.2, and 6.8 at 96 hours in the 0, 0.50, 1.0, 50, 500, and 5000 mg/L groups, respectively.
Reference:	DuPont Co. (1993). Unpublished Data, Haskell Laboratory Report No. 496-93, "Static Acute 96-Hour Screening Test to Fathead Minnows" (July 13).
Reliability:	Medium because a suboptimal study design with nominal concentrations was used for testing.

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	39,170 mg/L ($\log_{10}$ Kow of -1.14)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	N-methylformamide
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210.
Reliability:	Estimated value based on accepted model.

Additional Reference for Acute Toxicity to Fish:

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

BASF AG (1987). Abt. Toxikologie, Unpublished Report No. 87/646 (cited in Verschueren, K. (2001). Handbook of Environmental Data on Organic Chemicals, 4th ed., John Wiley & Sons, Inc., New York).

4.2 Acute Toxicity to Invertebrates

Type:	**24 and 48 EC_{50}**
Species:	*Daphnia magna Straus*
Value:	> 500 mg/L

Method: The procedures used in the test were based on the recommendations of the following guideline: EEC guideline 79/831/ECG, appendix V, part C.

The test containers were 20 mL flat-bottom test tubes. The test volume was 10 mL. There were 5 test animals per test tube with a total of 20 test animals per concentration (4 test tubes per concentration). The nominal concentration of the stock solution was 500 mg/L. The stock solution was diluted with water to obtain the test concentrations of 0, 62.5, 125, 250, and 500 mg/L.

The ability to swim was used in this test as a substitute criterion for liveability. Animals were considered unable to swim if, after knocking on the test vessels, no swimming movements occurred within 15 seconds. Swimming ability was assessed at 0, 3, 6, 24, and 48 hours.

Temperature measurements were recorded after 0, 24, and 48 hours in a separte vessel next to the test vessels. pH and O_2 measurements were recorded after 0 and 48 hours in 1 test duplicate per concentration.

GLP: No

Test Substance: N-methylformamide, purity 99.5%

Results: 24-48 EC_0 = 500 mg/L
24-48 EC_{50} > 500 mg/L
24-48 EC_{100} > 500 mg/L

The test temperature was reported as 21.5ºC.

The pH was 8.1, 8.3, 8.2, 8.2, and 8.1 at 0 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The pH was 8.0, 8.1, 8.1, 8.1, and 8.0 at 48 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The oxygen content was 9.1, 9.2, 9.0, 9.0, and 9.1 mg/L at 0 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The oxygen content was 8.4, 8.5, 8.5, 8.4, and 8.4 mg/L at 48 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively.

Conductivity was measured as 650 µS/cm and total hardness was measured as 2.68 mmol/L in the test water.

Reference: BASF AG (1989). Unpublished Report, Project No. 1/1451/2/88, "Determination of the Acute Toxicity of Monomethylformamide in the Water Flea, *Daphnia magna* Straus" (March).

Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.

Type: **48-hour EC_{50}**
Species: Daphnid
Value: 33,787 mg/L (log_{10} Kow of -1.14)
Method: Modeled
GLP: Not Applicable
Test Substance: N-methylformamide
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210.
Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type: **72-hour EC_{10-90}**
Species: *Scenedesmus subspicatus*
Value: > 8 g/L
Method: The procedures used in the test were based on the recommendations of the following guideline: DIN 38 412/9.

The concentrations tested were 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 42.4 g/L. Four duplicate experiments were run for each concentration and control. Room temperature was maintained at 20±1ºC. The experiment was conducted under continuous illumination. The cell concentrations were determined after 0, 24, 48, and 72 hours using a fluorimeter. The pH value was measured at the beginning and the end of the study in the control, and at all concentrations. The algae were kept in suspension by shaking twice daily on a test tube shaker. The stock solutions were prepared in a graduated cylinder at a concentration of 104.8 g/L with sterile twice distilled water. No solvent was used.

The computer evaluation of the inhibition was carried out using the probit method. The rate-related inhibition could not be evaluated by computer using the probit method. After 96 hours, the algal cell count was no longer doubled, and the

experiment was ended.

GLP: No Data

Test Substance: N-methylformamide, purity not reported

Results: Growth inhibition results (0-72 hours):

H_B 10_{72}: 13.88 g/L
H_B 50_{72}: 17.30 g/L
H_B 90_{72}: 21.57 g/L

Rate-related inhibition (0-72 hours):

Hμ 10_{72}: ---
Hμ 50_{72}: ---
Hμ 90_{72}: ---
Concentration of lowest inhibition (83.75%): 20.50 g/L
Concentration of highest inhibition (100%): 32.80 g/L
Growth promotion: 12.8 g/L

The pH was 8.2, 8.2, 8.1, 8.1, 8.1, 8.1, 8.1 at 0 hours for the 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 52.4 g/L groups, respectively. The pH was 8.7, 8.7, 8.6, 8.8, 8.6, 8.3, 6.1 at 72 hours for the 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 52.4 g/L groups, respectively.

Additional results are shown in the table below.

Concentration	Biomass	Growth rate	Inhibition in %	Rate-related inhibition in %
g/L	B	μ	HB	Hμ
0	1.11	1.01	-15.01	-3.62
5.0	1.27	1.04	-6.88	-1.66
8.0	1.18	1.02	-2.26	-3.54
12.8	1.13	1.04	83.75	55.23
20.5	0.18	0.45	100	137.97
32.8	---	-0.38	100	---
52.4	---	---	---	---

Reference: BASF AG (1992). Unpublished Report, Report No. 01/90/139, "Toxicity of Monomethylformamide on Green Algae" (March).

Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.

Type: **96-hour EC_{50}**

Species: Green algae

Value: 17,630 mg/L (log_{10} Kow of -1.14)

Method: Modeled

GLP: Not Applicable

Test Substance: N-methylformamide

Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210.
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral LD_{50}**
Species/Strain:	Rat/strain not reported
Value:	4000 mg/kg
Method:	No specific test guideline was reported.
GLP:	No
Test Substance:	N-methylformamide, purity not reported
Results:	No additional data.
Reference:	Thiersch, J. B. (1962). J. Reprod. Fertil., 81(4):219-220.
Reliability:	Not assignable because limited study information was available.

Type:	**Oral LD_{50}**
Species/Strain:	Female rats/Long Evans
Value:	7077 mg/kg
Method:	No specific test guideline was reported.
GLP:	No
Test Substance:	N-methylformamide, purity not reported
Results:	No additional data.
Reference:	Thiersch, J. B. (1971). Mason & Cie, Paris, 95-113.
Reliability:	Not assignable because limited study information was available.

Type:	**Oral LD_{50}**
Species/Strain:	Female mice/BALB/c
Value:	2600 mg/kg (95% confidence limits 2031-3328 mg/kg)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. At least 5 dose levels were employed. Mortality was assessed on day 30 post-administration. Lethal dose values

	and their 95% confidence limits were computed according to the method of Litchfield, J. T. and F. Wilcoxon (1949). J. Pharmacol. Exp. Ther., 96:99.
GLP:	No Data
Test Substance:	N-methylformamide, purity not reported
Results:	LD_{10} = 1800 mg/kg (95% confidence limits 1200-2700 mg/kg)
Reference:	Langdon, S. P. et al. (1985). Toxicology, 34(2):173-183.
Reliability:	Medium because a scientifically defensible study design was used, but limited study data were available.

Type:	**Oral LD_{50}**
Species/Strain:	Adult mice/Dub/ICR
Value:	2650 mg/kg
Method:	No specific test guideline was reported.
GLP:	No
Test Substance:	N-methylformamide, purity not reported
Results:	Time of death was between 1 – 8 days.
Reference:	Welsh, J. J. (1979). Diss. Abstr. Int. B, 40(2):549.
Reliability:	Not assignable because limited study information was available.

Additional Reference for Acute Oral Toxicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Laitarenko, G. N. et al. (1992). Gig. Sanit., (2):30-32 (CA 118:2071).

Type:	**Acute Inhalation Rangefinding Study**
Species/Strain:	Male rat/Crl:CD®
Value:	ALD > 10.76 mg/L
Method:	No specific test guideline was reported. Rats were exposed to the test substance in a single 4-hour exposure. Body weights and clinical signs were assessed. Six rats were exposed in each rangefinder group. Exposure concentrations were 0.69, 1.0, 5.6, and 10.76 mg/L.
GLP:	No
Test Substance:	N-methylformamide, purity 99.5%
Results:	No deaths occurred during the rangefinding study. Rats exposed to 5.6 and 10.76 mg/L showed severe weight loss followed by weight gain. Gasping was observed during exposure in 2/6 rats in the 10.76 mg/L group.
Reference:	DuPont Co. (1982). Unpublished Data, "Rangefinding Inhalation Study."

Reliability: Medium because a suboptimal study design was used.

Type: **Dermal ALD**
Species/Strain: Pregnant rat/ChR-CD, Sprague-Dawley
Pregnant rabbit/New Zealand White
Exposure Time: Not reported
Value: Rat: 11,000 mg/kg
Rabbit: 1500 mg/kg
Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats weighed 220-250 grams and rabbits weighed 4 kg at study start. MMF was applied with a syringe to the back skin (thoracic vertebral area) after the hair had been clipped. In determining the ALD, one animal was used per dose level with a factor of 1.5 between dose levels. Rats were treated on day 11 and rabbits were treated on day 15 of gestation. Surviving rats were sacrificed on day 21 and surviving rabbits on day 30 of gestation.
GLP: No
Test Substance: N-methylformamide, commercial grade with <2% impurities
Results: No additional data.
Reference: DuPont Co. (1966). Unpublished data, "Skin Application During Specific Gestation Days" (June).

Stula, E. F. and W. C. Krauss (1977). Toxicol. Appl. Pharmacol., 41:35-55.
Reliability: Medium because a suboptimal study design was used. Pregnant animals were used in the study and limited study information was available.

Additional References for Acute Dermal Toxicity: None Found.

Type: **Dermal Irritation: No Data**

Type: **Dermal Sensitization: No Data**

Type: **Eye Irritation**
Species/Strain: Rabbits/ New Zealand White
Method: The procedures used in the test were based on the recommendations of OECD Guideline 405: Acute Eye Irritation/Corrosion (May 12, 1981).

MMF (100 µL) was applied to the lower conjunctival sac of six rabbits. The Draize scoring criteria was used when eyes

were evaluated at 4, 24, 48, 72, and 96 hours post-instillation. One drop of 2% sodium fluorescein was applied to the eyes before visual scoring of percentage corneal damage.

GLP: No Data

Test Substance: N-methylformamide, purity not reported

Results: MMF was given an EEC classification of "Irritating to eyes" due to mean scores over 24/48/72 hours for conjunctivitis greater than two.

	Time after application (in hours):				
	4	24	48	72	96
Mean score of Conjunctivitis (max of 3)	2.3	2.4	2.2	2.0	1.5
Mean score of chemosis (max of 4)	2.0	1.2	1.0	0.3	0.0
Mean score of iritis (max of 2)	1.0	0.7	0.0	0.0	0.0
Mean score of corneal opacity (max of 4)	1.0	0.8	0.7	0.3	0.0
Mean surface of corneal damage (100% max)	43	60	32	11	10

Reference: Jacobs, G. A. (1992). Acute Toxic. Data, 1(3):187-188.

Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for Eye Irritation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Conquet, P. et al. (1977). Toxicol. Appl. Pharmacol., 39(l):129-139 (CA86:115698b).

5.2 Repeated Dose Toxicity

Type:	**2-Week Inhalation Study**
Species/Strain:	Rats/Crl:CD®
Sex/Number:	Male/15 per group
Exposure Period:	2 weeks
Frequency of Treatment:	6 hours/day, 5 days/week
Exposure Levels:	0, 0.1, 0.3, 1.0 mg/L (0, 50, 132, 402 ppm)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats were 7-8 weeks old and weighed 207-242 grams at study start.

Inhalation exposures were nose-only. The liquid test material was syringe-driven into a heated (180ºC) 3-neck flask, where it was flash evaporated. Unheated dilution air passed through the flask and carried vapors to the exposure chamber. Atmospheric concentrations were periodically measured and analyzed via gas chromatography. Chamber temperature was monitored with a thermometer during each exposure.

All rats were weighed and observed daily (excluding weekends) throughout the exposure and recovery periods.

Clinical laboratory measurements were made on urine samples collected overnight following the 9th exposure and the 13th day of recovery. Blood samples were taken from the rats' tails after the 10th exposure and the 14th day of recovery. Approximately 13 hematological parameters were measured or calculated, 7 clinical chemistry parameters were measured or calculated, and 12 urine chemistry parameters were measured or examined.

After the 10th exposure, 5 rats from each group were selected at random and sacrificed for gross and histopathological examination. Five of the remaining rats were sacrificed on the 14th day of recovery for identical examination. Approximately 25 organs and/or tissues were saved for microscopic examinations. Seven organ weights (heart, liver, lungs, kidneys, spleen, testes, and thymus) were recorded. Organs and tissues examined included adrenal

glands, thyroid gland, esophagus, stomach, duodenum, pancreas, jejunum, ileum, cecum, colon, liver, spleen, thymus, mediastinal lymph nodes, eye, brain, trachea, heart, nose, urinary bladder, lungs, sternum, kidneys, testes, and epidiymides.

Of the 15 rats/group exposed to the test material, 5/group were used solely for collection of urine samples for MMF analysis. Urine samples were collected overnight from these rats on exposure days 1, 4, and 9 and on recovery days 3, 6, and 13. Samples were analyzed by gas chromatography.

The body weight, hematologic, and clinical chemistry data were statistically analyzed by a one-way analysis of variance. Least significant differences and Dunnett tests were used to compare MMF-treated rats with the controls when the ratio of variance indicated a significant, among to within group variation.

GLP: No

Test Substance: N-methylformamide, purity 99.5%

Results: Mean measured exposure concentrations were 0.12, 0.32, and 0.97 mg/L for the 0.1, 0.3, and 1.0 mg/L groups, respectively. Chamber temperature was maintained at 27-34ºC.

Clinical observations of rats exposed to 0.12 mg/L were indistinguishable from controls throughout the study. Rats exposed to 0.32 mg/L had significantly lower body weights during the first week and the latter part of the second week of exposure. Gain during the recovery phase was parallel to that of controls. Rats exposed to 0.97 mg/L had significantly lower body weights throughout the study with severe weight depression during the exposure phase and weight gain at a rate parallel to that of controls during the recovery phase.

Clinical chemistry measurements made at the end of the exposure period showed no compound-related effects in rats exposed to 0.12 mg/L. Rats exposed to 0.32 and 0.97 mg/L had increased serum cholesterol concentrations. Rats exposed to 0.97 mg/L also had decreased serum urea nitrogen concentrations, decreased serum alkaline phosphatase activities, and increased serum ALT/GPT and AST/GOT activities. These changes were interpreted to be evidence of treatment-related effects on the integrity and function of hepatic tissue. All compound-related effects observed at the end of the exposure period were absent

14 days later.

Pathologic examination revealed no compound-related macroscopic lesions in any rats. Microscopically, there were compound-related effects following exposure in the livers of rats exposed to 0.32 and 0.97 mg/L. Lesions included pale cytoplasm, increase in the number of mitotic figures, and cytoplasmic lipid vacuolation. These changes were interpreted as being degenerative and regenerative in nature. Fourteen days following exposure, partial recovery at the high level and complete recovery at the intermediate level had occurred. Incidence levels of the microscopic findings mentioned above can be found in the following table.

	Exposure Concentration (mg/L)			
	0	**0.12**	**0.32**	**0.97**
Pale cytoplasm	0/5(a) 0/5(b)	0/5(a) 0/5(b)	5/5(a) 1/5(b)	5/5(a) 5/5(b)
Increase in mitotic figures	0/5(a) 0/5(b)	0/5(a) 0/5(b)	3/5(a) 0/5(b)	2/5(a) 0/5(b)
Cytoplasmic lipid vacuolation	0/5(a) 0/5(b)	0/5(a) 0/5(b)	1/5(a) 0/5(b)	5/5(a) 0/5(b)
a = incidence following exposure period b = incidence following recovery period				

A comparison of organ weights between test rats and controls showed no changes in rats exposed to 0.12 mg/L. Following 10 exposures, mean absolute weights of the heart, lung, spleen, and thymus were significantly lower in rats exposed to 0.97 mg/L than in controls. On a relative basis, rats exposed to 0.32 and 0.97 mg/L had increased liver/body weight ratios. Fourteen days following exposure, there were no differences between test rats and controls. Although relative testes weights were increased at 0.97 mg/L following 10 exposures, no microscopic findings were observed in the testes, and no differences in testes weight were found fourteen days following exposure.

MMF was excreted in a dose-dependent fashion in the urine of exposed rats. It was detected in the urine of rats exposed to 0.32 and 0.97 mg/L on day 1 and in rats exposed to 0.12 mg/L on day 4. In all test groups, urinary levels of MMF generally increased throughout the exposure period and then decreased throughout the recovery period. At the end of the recovery period, MMF was still detectable in rats

exposed to 0.32 and 0.97 mg/L, but not in rats exposed to 0.12 mg/L.

The no-effect level of the study was determined to be 0.12 mg/L. The intermediate and high levels exhibited dose-dependent effects with primary effects on the liver.

Reference: DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report 162-83, "Subacute Inhalation Toxicity Study in Rats" (May 3) (also cited in TSCA Fiche OTS0571678).

Kennedy, G. L., Jr. et al. (1990). Fundam. Appl. Toxicol., 14(4):810-816.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1971). Unpublished Data, Haskell Laboratory Report 75-71 (cited in TSCA Fiche OTS0520900, OTS0555847, and OTS0558318).

Chanh, P.-H. et al. (1971). Therapie, 26(3):409-424 (CA 75:107799t).

Supporting Data: DMF

Type:	**2-Week Inhalation Study**
Species/Strain:	Rats/Not reported
Sex/Number:	Not reported/Not reported
Exposure Period:	10 days
Frequency of Treatment:	0.5 or 6 hours/day
Exposure Levels:	0, 91, 1104, or 91 and 841 ppm
Method:	No specific test guideline was reported.

Four test groups were employed in this preliminary study. Group I received 91 ppm, 6 hours/day for 10 days. Group II received 1104 ppm, 0.5 hours/day for 10 days. Group III received 91 ppm, 6 hours/day for 10 days followed by a 841 ppm exposure for 0.5 hours at the end of the 10th exposure. The CT (concentration, ppm x time, hours) for the groups were 5460, 5520, and 5880 ppm x hours for Groups I, II , and III, respectively. The control group received air for 6 hours/day for 10 days.

GLP: No
Test Substance: DMF, purity not reported
Results: Liver weights of the test rats were elevated and the liver to body weight ratios of the test groups were statistically greater than the controls.

	Exposure Group			
	I	II	III	Control
Liver to body weight ratio (%)	4.39	4.36	4.57	4.02
Liver weight range (g)	13.3-18.9	13.0-17.6	13.7-18.7	12.0-17.1

Reference: Clayton, J. W. et al. (1963). Am. Ind. Hyg. Assoc. J., 24:144-154.
Reliability: Medium because a sub-optimal study design was used. Study was only a preliminary study and complete study details were not reported.

Type: **90-Day Inhalation Study**
Species/Strain: Rats/Fisher 344
Mice/B6C3F1
Sex/Number: Male and female/30 rats per sex per group; 10 mice per sex per group
Exposure Period: 13 weeks
Frequency of Treatment: 5 days/week, 6 hours/day
Exposure Levels: 0, 50, 100, 200, 400, and 800 ppm
Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats and mice were exposed via whole-body inhalation. Analytical concentrations were determined via infrared analysis.

Rats were 51 days of age at the first exposure. Mice were 46 days of age at first exposure. For the rats, each study group was divided into three subgroups of 10 rats per sex each. The three subgroups consisted of a base study group, a cardiovascular group, and a renal function group.

Animals were observed twice daily for mortality and moribundity. Body weights were measured weekly throughout the study and at necropsy.

Sperm morphology and vaginal cytology evaluations were performed on rats and mice exposed to 0, 50, 200, and 800 ppm DMF. Sperm morphology was evaluated at necropsy. Vaginal cytology was done by vaginal lavage with saline during the 2 weeks prior to necropsy.

Clinical pathology evaluations were conducted on cardiovascular study rats at 4 and 23 days and on base-study rats at 13 weeks. Urinalysis was performed on 5 rats/sex in the 0, 50, 200, and 800 ppm groups. Kidney histology was performed on these animals.

Blood pressure and electrocardiograms were measured within 24 hours of the last DMF exposure in the cardiovascular group rats. The animals were killed and the heart removed for microscopic evaluation.

At study termination, rats in the base study and the renal function groups and mice were killed and complete necropsies were performed. Examination for gross lesions was conducted and liver, thymus, kidneys, testicles, heart, and lung weights were recorded. The liver was microscopically examined in all dose groups. In addition, approximately 35 other organs or tissues were examined histologically in the control and high-dose groups.

Organ, body weight, blood pressure, and electrocardiographic data were analyzed using the parametric comparisons procedures of Williams, D. A. (1971). Biometrics, 27:103-117, Williams, D. A. (1972). Biometrics, 28:519-531, and Dunnett, W. (1955). J. Amer. Stat. Assoc., 50:1095-1121. Clinical chemistry and hematology data were analyzed using nonparametric multiple comparisons methods of Shirley, E. (1977). Biometrics, 33:386-389 and Dunn, O. J. (1964). Technometrics, 6:241-252. Jonckheere's test was used to assess significance of dose-response trends, and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). The outlier test of Dixon, W. and F. Massey (1951). Introduction to Statistical Analysis, pp. 145-147, McGraw Hill, NY was used to detect extreme values.

GLP: Yes

Test Substance: DMF, purity > 99%

Results: The analytical concentrations were 0, 50.2, 98.6, 198.1, 401.3, and 804.6 ppm for the 0, 50, 100, 200, 400, and 800 ppm groups, respectively.

Rats

There was no substance-related mortality. Body weight gains were reduced by approximately 47-65% in rats exposed to 800 ppm and to a lesser degree in the animals of the 400 ppm group.

Evidence for hepatocellular injury was seen as early as day 4 as increases in activities of liver-specific enzymes (e.g. ALT, SDH, and ICDH) in the serum of both sexes at 200 – 800 ppm DMF. Serum cholesterol levels were increased in all exposed rats at all time points (4, 24, and 91 days).

Relative liver weights were increased in the males at 100 ppm and above and at all concentrations of the females. Minimal to moderate centrilobular hepatocellular necrosis was seen in both sexes at 400 and 800 ppm and pigment accumulation (hemosiderin and lipfuscin) in macrophages and Kupffer cells was found in both sexes at 800 ppm.

Prolonged diestrus was observed in 7 of 10 females exposed at 800 ppm (i.e. at a concentration that produced hepatotoxicity and reduced body weight gain).

Relative testis weights were increased at 400 and 800 ppm DMF; however, no microscopic findings or adverse effects on sperm density or motility were observed.

Mice

Five male mice died of undetermined causes during the study; 3 of these were in the lowest exposure group, suggesting that exposure to DMF was not involved. All female mice survived the 13-week exposure period. No exposure-related clinical signs were observed. Reduced body weight gain was noted in the 800 ppm female mice.

Relative and/or absolute kidney and lung weights were variably increased in all exposed groups of females. Both absolute and relative thymus weights were decreased in the 50 ppm male mice. This finding was not considered biologically significant. Absolute liver weights were moderately increased in the 200-800 ppm males and 50-800 ppm females. Relative liver weights were increased in

both sexes at all exposure levels.

No significant changes in the reproductive system evaluations were observed in male mice. In females, there was a significant trend toward an increase in the estrous cycle length. The animals in the 200 ppm group spent significantly more time in the stages of estrus and diestrus than did the controls.

Centrilobular hepatocellular hypertrophy (minimal to mild) was found in all groups of male mice and in female mice exposed to = 100 ppm.

Reference: NTP (1992). Technical Report No. 22, "Toxicity Studies on N,N-dimethylformamide in F344/N Rats and B6C3F1 mice (13-Week Inhalation Studies)" PB93191936 (November).

Reliability: High because a scientifically defensible or guideline method was used.

5.3 Developmental Toxicity

Study No. 1

Species/Strain: Rats/Crl:CD (SD)COBS
Rabbits/New Zealand White

Sex/Number: Female rats/25 per group
Female rabbits/20 per group

Route of Administration: Oral gavage

Exposure Period: Rats/Gestation days 6-15
Rabbits/Gestation days 6-18

Frequency of Treatment: Daily

Exposure Levels: Rats/0, 1, 5, 10, 75 mg/kg
Rabbits/0, 5, 10, 50 mg/kg

Method: The procedures used in the test were based on the recommendations of the following guideline: EPA (1986). Developmental toxicity risk assessment guidelines, Fed. Regist., 51:34028-34040.

All animals were housed individually in stainless steel cages suspended over cage board in environmentally controlled rooms. Food and tap water were available *ad libitum*. A 12-hour light/12-hour dark photoperiod was provided. Room temperature was maintained at 72±4ºF for rats and 68±5ºF for rabbits. Relative humidity was maintained at 55±15%.

Female rats (226-289 g) were naturally mated with adult males

from the same strain. Female rabbits (ca. 3-4.1 kg) were artificially inseminated. The presence of a copulatory plug or a positive vaginal smear was designated as gestation day 0.

Human chorionic gonadotropin was administered to the rabbits in the marginal ear vein immediately following the insemination. The day of insemination was designated as gestation day 0.

Dose suspensions were prepared in deionized water every 3 days for rats and every 7 days for rabbits. Animals in the control group received deionized water.

Maternal body weights and physical signs were monitored throughout the study. Food consumption was measured on designated weigh days (rats) or daily (rabbits).

Cesarean sections were performed on rats and rabbits on gestation days 20 and 29, respectively. Uterine and fetal examinations were conducted.

Individual rat fetuses were weighed and examined externally for anatomical anomalies and sex determination. Approximately half of the fetuses from each litter underwent a visceral examination by the method of Wilson, J. G. (1965). In Teratology Principles and Techniques, University of Chicago Press, Chicago. The remaining fetuses were eviscerated, fixed in ethanol, and stained with alizarin red S for skeletal evaluation.

All rabbit fetuses were individually weighed and examined for external and visceral anomalies by the method of Staples, R. E. (1974). Teratology, 9:A37-A38. Sex was determined during the internal examination. Rabbit fetuses were examined for cephalic soft tissue anomalies by a mid-cranial section. Rabbit fetuses were fixed in ethanol and stained with alizarin red S for skeletal examination.

Continuous data including fetal and maternal body weights, maternal body weight gain, maternal food consumption, number of fetuses, implantations, and corpora lutea were analyzed via one-way analysis of variance. When appropriate, group comparisons were conducted using Dunnett's analysis. Discontinuous data were analyzed using the $?^2$ test for fetal sex ratios, Mann-Whitney U test for resorptions, and a one-tailed Fisher exact test for the number of fetal variations and

malformations.

GLP: No Data

Test Substance: N-methylformamide, purity > 99%

Results: Dose suspensions were determined analytically by gas chromatography to be accurately prepared, homogeneous, and stable for 7 days at room temperature.

Rats

There were no treatment-related maternal deaths, clinical signs, or gross pathologic changes in treated dams. Body weight gain and food consumption were depressed in rats given 75 mg/kg.

The number of corpora lutea, implantations, and fetal sex ratio were similar in all experimental groups. Fetal viability was reduced at 75 mg/kg in rats due to a significant increase in the number of early resorptions. Fetal weights were depressed in the 75 mg/kg group. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

	Concentration (mg/kg)				
Observation:	**0**	**1**	**5**	**10**	**75**
No. gravid females with live litter:	25	25	25	25	22
Corpora lutea/dam:	17.7	17.3	16.5	16.6	16.5
Pre-Implantation loss/litter:	2.0	1.2	1.6	0.6	1.1
Post-Implantation loss/litter:	0.9	1.0	0.8	1.0	3.1
Total number of live fetuses:	370	375	351	373	269
Live Fetuses/litter	14.8	15.0	14.0	14.9	12.2
Mean Fetal Weight (g):	3.5	3.5	3.5	3.4	2.9
Sex Ratio (M/F):	204/166	196/179	178/173	185/188	127/142
Number of malformed fetuses/number of litters affected:	0/0	0/0	1/1	3/3	150/21
NR = Not Reported					

At 75 mg/kg, over 50% of the fetuses were malformed. A

significant increase in the incidence of malformations including cephalocele and sternoschisis was observed in fetuses from the 75 mg/kg group. The isolated incidences of the malformations of gastroschisis, right-sided aortic arch, and sternebrae fused at 75 mg/kg were considered to be spontaneous in nature.

In addition, a developmental delay was indicated by reduction of fetal weight and by a significant increase in the occurrence of incomplete ossification of various skeletal structures, including reduced ossification of general non-ossification of the skull, 13th rib(s), and sternebrae.

Fetal data from the 1, 5, and 10 mg/kg groups were comparable to the control group. There were 3 fetuses malformed in the 10 mg/kg group; however, these malformations were considered to be spontaneous in nature because of the single incidence and lack of a dose-response relationship. The number of fetuses/litter with reduced ossification of the 13th rib was slightly increased at 10 mg/kg compared to control values. However, the variation occurred at an incidence comparable to the historical control population of the testing facility. For the historical control population, 0.0 to 3.6% of the fetuses were affected.

A summary of fetal malformations and variations in rats is provided in the table below. Incidences of findings are presented as number of fetuses affected/number of litters affected unless otherwise indicated.

	Concentration (mg/kg)				
	0	**1**	**5**	**10**	**75**
External Exam*	370/25	375/25	351/25	373/25	269/22
Visceral Exam*	188/25	189/25	179/25	185/25	134/22
Skeletal Exam*	182/25	187/25	172/25	189/25	135/22
Malformations					
Cephalocele	0/0	0/0	0/0	0/0	144/20
Sternoschisis	0/0	0/0	0/0	0/0	9/5
Anal atresia and filamentous tail	0/0	0/0	0/0	1/1	0/0
Gastroschisis	0/0	0/0	0/0	0/0	1/1
Micrognathia	0/0	0/0	0/0	1/1	0/0
Open eyelids	0/0	0/0	0/0	1/1	0/0
Multiple anomalies	0/0	0/0	0/0	1/1	0/0
Right-sided aortic arch	0/0	0/0	0/0	0/0	1/1

Fused sternebrae	0/0	0/0	0/0	0/0	1/1
Vertebral anomalie	0/0	0/0	1/1	0/0	0/0
Atlas-occipital defect	0/0	0/0	0/0	1/1	0/0
Variations					
Reduced ossification of the skull	8/5	7/4	5/3	5/3	66/15
Unossified sternebrae no. 5 and/or no. 6	6/3	6/5	6/5	13/5	36/13
Reduced ossification of 13th rib	2/2	3/2	0/0	8/6	12/8
Unossified sternebrae nos. 1, 2, 3, and/or 4	0/0	1/1	0/0	1/1	5/5
Distended ureter	7/4	15/11	13/8	12/6	19/10
Malaligned sternebrae	38/21	48/21	38/20	55/24	58/19
14th rudimentary rib	6/4	2/2	3/3	2/1	0/0
Bent ribs	4/4	2/2	4/3	0/0	1/1
7th cervical rib	4/3	0/0	0/0	3/3	0/0
Sternebrae with thread-like attachment	0/0	0/0	0/0	0/0	1/1
27 presacral vertebrae	0/0	0/0	1/1	0/0	0/0
Unossified pubis	0/0	0/0	0/0	0/0	1/1
Unossified hyoid	1/1	1/1	0/0	0/0	1/1
* = Number of fetuses examined/number of litters					

Rabbits

There were no treatment-related maternal deaths, clinical signs, or gross pathologic changes in treated dams. Maternal deaths of 1 female in the 5 and 10 mg/kg groups, and 2 females in the 50 mg/kg group were the result of dosing trauma. No other deaths occurred. Body weight gain and food consumption were depressed in rabbits given 50 mg/kg.

The mean number of corpora lutea was reduced at 50 mg/kg; however, the authors considered this reduction to be of no toxicological significance because the mean number of corpora lutea in the control group exceeded the historical control range for the testing facility (mean 12.0, range, 10.9-12.3). In addition, historical control data from MARTA for this

parameter has been determined to be 10.5 for New Zealand white rabbits (Lang, P. L. (ed). (1993). Historical Control Data for Developmental and Reproductive Toxicity Studies Using the New Zealand White Rabbit, Hazleton Research Products, Inc., Denver, Pa.).

The number of implantations and sex ratio were unaffected by treatment. Fetal viability was reduced at 50 mg/kg in rabbits as evidenced by post-implantation loss. Fetal body weight was also reduced at 50 mg/kg. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

	Concentration (mg/kg)			
Observation:	**0**	**5**	**10**	**50**
No. gravid females with live litter:	19	17	16	15
Corpora lutea/doe:	13.4	11.9	10.9	9.6
Pre-Implantation loss/litter:	6.8	4.9	4.2	3.2
Post-Implantation loss/litter:	0.4	0.5	0.5	1.5
Total number of live fetuses:	NR	NR	NR	NR
Live Fetuses/litter	6.2	6.5	6.2	5.0
Mean Fetal Weight (g):	46.1	44.2	46.1	37.0
Sex Ratio (% M/F):	53/64	50/60	50/49	38/47
No. of malformed fetuses/number of litters affected:	5/5	4/3	3/3	85/15
NR = Not Reported				

Treatment-related malformations observed at 50 mg/kg included gastroschisis, cephalocele, domed head, flexed paw, and skull and sternum anomalies. Although there was an increased incidence of the developmental variation of bent hyoid at 50 mg/kg, this finding was not statistically significant. Fetal data for the 5 and 10 mg/kg were comparable to control data. A summary of fetal malformations and variations in rabbits is provided in the table below. Incidences of findings are

presented as number of fetuses affected/number of litters affected unless otherwise indicated.

	Concentration (mg/kg)			
	0	**5**	**10**	**50**
External Exam*	117/19	110/17	99/16	85/15
Visceral Exam*	117/19	110/17	99/16	85/15
Skeletal Exam*	117/19	110/17	99/16	85/15
Malformations				
Gastroschisis	0/0	0/0	0/0	84/15
Cephalocele	0/0	0/0	0/0	14/5
Domed head	0/0	0/0	0/0	11/4
Skull anomaly	0/0	0/0	0/0	46/12
Sternum anomaly	0/0	0/0	0/0	75/14
Micrognathia	1/1	0/0	0/0	0/0
Flexed paw	0/0	0/0	0/0	10/3
Hydrocephaly	1/1	0/0	0/0	0/0
Kidney and/or ureter agenesis	0/0	0/0	0/0	1/1
Rib anomaly	1/1	0/0	0/0	0/0
Sternebrae fused	1/1	2/2	1/1	3/1
Sternebrae misaligned (severe)	0/0	0/0	1/1	0/0
Vertebral anomaly	2/2	2/1	0/0	1/1
Extra site of ossification anterior to sternebra no. 1	0/0	0/0	1/1	0/0
Variations				
Major blood vessel variation	3/3	0/0	1/1	1/1
Hemorrhagic ring around the iris	3/3	3/3	1/1	0/0
Retrocaval ureter	5/4	2/2	1/1	3/3
Gallbladder absent or small	0/0	1/1	0/0	2/2
13th full rib	55/16	43/15	54/15	51/13
Sternebrae no.5 and/or no. 6 unossified	13/4	3/3	3/1	1/1

13th rudimentary rib	14/9	17/12	10/6	17/10
27 presacral vertebrae	22/11	10/8	21/10	23/6
Sternebrae with threadlike attachment	1/1	2/2	4/3	1/1
Hyoid arches bent	6/6	8/5	3/3	22/9
Sternebrae malaligned (slight or moderate)	19/11	16/8	11/8	3/3
Accessory skull bone	0/0	2/2	0/0	1/1
Hyoid body and/or arches unossified	0/0	1/1	0/0	0/0
7th sternebrae	0/0	0/0	2/2	0/0
* = Number of fetuses examined/number of litters				

The lowest-observed-adverse-effect levels for maternal and developmental toxicity in the rat and rabbit were 75 and 50 mg/kg, respectively. The no-observed-adverse-effect level for maternal and developmental toxicity in the rat and rabbit was 10 mg/kg.

Reference: Kelich, S. L. et al. (1995). Fundam. Appl. Toxicol., 27(2):239-246 (also cited in TSCA Fiche OTS0544113).

Liu, S. L. et al. (1989). Teratology, 39(5):466 (Abstract P70).

Reliability: High because a scientifically defensible or guideline method was used.

Study No. 2

Species/Strain: Rats/Crl:CD®BR

Sex/Number: Female/25 per group

Route of Administration: Nose-only inhalation

Exposure Period: Gestation Days 7-16

Frequency of Treatment: 6 hours/day

Exposure Levels: 0, 15, 50, 150 ppm

Method: The procedure used in the test were based on the recommendations of the following guidelines:

US EPA (1985). TSCA Test Guidelines, Federal Register, 50, No. 188, September 27; US EPA (1987). Revised 52, No. 97, May 20 and

US EPA (1984). New and Revised Health Effects Test Guidelines, October, US EPA, Washington, DC.

Female rats were 63 days old upon arrival and weighed between 170.1 to 218.3 grams on the day after arrival. Mature male rats of the same strain and from the same supplier were 84 days old upon arrival and weighed between 308.9 to 376.4 grams prior to mating. Animals were individual housed, except during mating, in stainless steel wire-mesh cages. Food and water were available *ad libitum*. Animal rooms were on a 12-hour light/12-hour dark cycle. Room temperature and relative humidity were targeted at 21-25°C and 40-60%, respectively.

Females were cohabited with males (1:1) until copulation was confirmed by the presence of a copulatory plug in the vagina or on the cageboard. The day that copulation was confirmed was designated as day 1 of gestation.

Test atmospheres of MMF were generated by vaporization. Conditioned, filtered houseline air was metered through glass reservoirs containing the liquid test material to generate MMF vapor. The reservoirs for the intermediate and high-level chambers were immersed in water baths and heated to approximately 21-29°C to increase the evaporation rate of the test material. The reservoir for the low level chamber was maintained at ambient temperature. Conditioned, filtered houseline air was added to dilute the vapor and to sweep the resulting vapor/air mixtures into the inlets of 150-L stainless steel and glass exposure chambers. Exposure chamber concentrations of MMF were controlled by varying the flow rates of generation or dilution air. To promote uniform distribution, the test mixtures were dispersed with baffles upon entering the exposure chambers. The control rats were exposed to conditioned, filtered houseline air only, using the same type of exposure chamber.

The atmospheric concentration of MMF in each test chamber was measured periodically by gas chromatographic analysis. Chamber temperatures, relative humidity, and chamber oxygen concentrations were also measured.

Rats were individually restrained in perforated, stainless steel cylinders fitted with conical nose pieces. The nose pieces were coated with parafilm to minimize droplet deposition on the rats' face or head. Each restrainer was inserted into a Plexiglas face plate on the exposure chamber such that only the rat's nose and

potentially part of the head could protrude into the chamber.

Dams were regularly monitored throughout gestation for body weight gain, feed consumption, and clinical signs. Cesarean sections were performed on Day 22G and the adult females were examined for gross anatomical abnormalities. Liver, thymus, and gravid uterus were removed and weighed. Additional parameters assessed included the number and relative position of nidations (live and dead fetuses, and early and late resorptions), empty uterine weight, and the number of corpora lutea.

Fetal weights and fetal sex were recorded. An external examination was conducted on all fetuses. Approximately half of the fetuses/litter were decapitated and examined for visceral examinations using the Staples technique (Staples, R. E. (1974). Teratology, 9:A37). The heads were fixed in Bouin's solution and examined. All fetuses underwent a skeletal examination.

Incidence of pregnancy, clinical observations, maternal mortality, and litters with total resorptions were analyzed via the Cochram-Armitage test for linear trend and the Fisher's exact test. Maternal weight, weight change, feed consumption, and maternal liver and thymus weights were analyzed with the linear combination of dose ranks from ANOVA and the Dunnett's test if the ANOVA was significant. Live fetuses, dead fetuses, resorptions, nidations, corpora lutea, fetal weight, and incidence of fetal alterations were analyzed with Jonkheere's test and the Mann-Whitney U test.

GLP: Yes

Test Substance: N-methylformamide, purity 99.9%

Results: The mean analytical concentrations for the 0, 15, 50, and 150 ppm groups were 0, 14.8, 51.5, and 150 ppm, respectively. The chamber temperatures ranged from 21-28, 23-29, 24-30, and 24-29ºC for the 0, 15, 50 and 150 ppm concentration levels, respectively. The mean percent relative humidity was 38-58, 44-68, 45-60, and 44-61% for the 0, 15, 50, and 150 ppm concentration groups, respectively. The chamber oxygen concentrations were approximately 21% for both test and control chambers throughout the study.

One compound-related death occurred on day 14 of gestation in the 150 ppm group. Exposure to 50 or 150 ppm produced significant increases in the number of dams exhibiting mild respiratory distress, characterized as wheezing and rattling, during and after the exposure period. Other clinical signs

observed were primarily attributable to the restraint conditions of the study, however, a possible decrease in grooming at 150 ppm may explain the persistence of some of these signs post-exposure. Maternal toxicity was also evident at the 150 ppm level as evidenced by significant adverse effects on body weight, food consumption, and absolute and relative thymus weights.

Embryolethality was evident by the significantly increased mean number of resorptions per litter seen in females exposed to 150 ppm of MMF. Correspondingly, the mean number of live fetuses per litter was significantly decreased at 150 ppm. Developmental toxicity was also evident as a significant reduction in mean fetal body weight at exposure levels of 50 and 150 ppm; a significant trend accompanied this finding. Relative to controls, the weight reduction at 50 ppm was regarded as slight. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

	Concentration (ppm)			
Observation:	**0**	**15**	**50**	**150**
Number pregnant	23/25	25/25	24/25	22/25
Corpora lutea:	15.3	15.6	15.1	15.4
Nidations:	14.5	14.2	13.6	14.2
Total No. of Fetuses:	NR	NR	NR	NR
Mean Resorptions:	0.9 T 0.9 E 0.0 L	0.6 T 0.6 E 0.0 L	0.8 T 0.8 E 0.0 L	2.0 T 2.0 E 0.0 L
Live Fetuses:	13.7 T 6.9 M 6.7 F	13.6 T 6.9 M 6.7 F	12.8 T 6.5 M 6.3 F	12.3 T 6.6 M 5.7 F
Mean Fetal Weight (g):	5.17 T 5.30 M 5.00 F	5.12 T 5.23 M 5.02 F	5.06 T 5.20 M 4.86 F	4.54 T 4.65 M 4.40 F
Sex Ratio (No. of Males/Total No.):	NR	NR	NR	NR
T = total, E = early, L = late M = male, F = female NR = Not Reported				

The incidence of structural malformations was significantly increased in fetuses from the 150 ppm exposure group. The malformations that primarily contributed to this increase were

subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. Additionally, developmental toxicity was evident by an exposure-related response in the incidence of fetuses with variations, specifically those due to growth retardation where a significant delay in skeletal ossification was seen in fetuses from the 150 ppm group. Although the overall incidence of developmental variations was not significantly different across groups, significance was noted for total skeletal variations consisting primarily of misaligned and fused sternebrae. A significant trend and increase relative to control values was also seen in the 150 ppm group for the mean percentage of fetuses per litter with variations due specifically to retarded development. This effect was attributable to delayed skeletal ossification. A summary of fetal malformations and variations in rats is provided in the table below. Incidences of findings are presented as number of fetuses affected/number of litters affected unless otherwise indicated.

	Concentration (ppm)			
	0	**15**	**50**	**150**
External Exam*	314/23	341/25	308/24	258/21
Visceral Exam*	164/23	177/25	160/24	148/21
Skeletal Exam*				
Malformations				
Abdomen - Gastroschisis	0/0	0/0	0/0	1/1
Head – Subcutaneous cyst	0/0	0/0	0/0	21/2
Tail - Vestigial	0/0	0/0	1/1	0/0
Kidney – No papilla	0/0	1/1	0/0	0/0
Brain – 3rd ventricle distended	0/0	0/0	0/0	1/1
Brain - Hydrocephaly	1/1	0/0	0/0	2/2
Brain – Lateral ventricle distended	0/0	2/2	1/1	4/2
Eye - Anophthalmia	0/0	0/0	0/0	1/1
Eye - Microphthalmia	0/0	0/0	0/0	3/1
Rib - Fused	0/0	0/0	0/0	9/2
Vertebrae - Fused	0/0	0/0	0/0	7/1

Vertebrae - Hemi	0/0	0/0	0/0	8/1
Total No. Affected with Malformations	**1/1**	**3/3**	**2/2**	**30/6**
Variations				
Sternebra – Misaligned (1)	1/1	3/2	2/2	2/2
Sternebrae – Misaligned (2+)	1/1	2/2	2/2	11/6
Sternebra - Fused	0/0	0/0	0/0	13/4
Total No. Affected with Skeletal Developmental Variations	**27/12**	**15/10**	**11/6**	**32/15**
Mean % Affected	**7.8**	**4.3**	**4.6**	**18.7**
Rib – Rudimentary Th13	6/5	4/4	8/5	28/10
Skull – Frontal partially ossified	0/0	0/0	0/0	7/6
Skull – Interparietal partially ossified	9/6	13/7	12/11	32/12
Skull – Parietal partially ossified	0/0	7/4	3/3	12/5
Skull – Squamosal partially ossified	2/2	4/3	2/2	8/4
Skull – Supraoccipital partially ossified	2/2	10/6	8/8	87/19
Skull – Supraoccipital unossified	0/0	0/0	0/0	9/4
Skull – Zygoma partially ossified	3/3	6/5	3/3	9/4
Vertebra – Dumbbelled centrum	8/8	8/5	16/8	26/12
Vertebra – Partially ossified	2/2	2/2	8/6	20/11

Total No. with Variations due to Retarded Development	**44/20**	**49/17**	**59/22**	**137/20**
Mean % Affected	**13.3**	**13.8**	**19.4**	**62.0**
* = Number of fetuses examined/number of litters				

Under the conditions of this study, maternal lethality and toxicity was demonstrated at 150 ppm of MMF and maternal toxicity remained evident as mild respiratory distress in the 50 ppm treated dams. Although to a lesser degree than at 150 ppm, developmental toxicity, expressed as a slight depression in mean fetal body weight was evident at 50 ppm. The NOEL for both the dam and the fetus was 15 ppm. Thus the conceptus was found to be sensitive only at exposure levels that were also toxic to the dams.

Reference: DuPont Co. (1988). Unpublished Haskell Laboratory Report No. 510-88, "Teratogenicity Study of N-methylformamide (MMF) in the Rat (December 21) (also cited in TSCA Fiche OTS0000617-1 and OTS0535910).

Rickard, L. B. and C. D. Driscoll (1990). Teratology, 41(5):586 (also cited in OTS02890617 (main study) and OTS05880617 (pilot study)).

Rickard, L. B. et al. (1995). Fundam. Appl. Toxicol., 28(2):167-176.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Developmental Toxicity:

MMF has been studied for its developmental toxicity by the oral, inhalation, dermal, intraperitoneal, subcutaneous, and *in vitro* routes of exposure. The 2 studies which represented the most standard study designs for developmental toxicity and which followed recommended EPA test guidelines were the studies chosen for detailed summarization. Of these studies, one was via the oral route of exposure and one was via the inhalation route of exposure. Both studies determined that the conceptus was sensitive to MMF only at exposure levels that were also toxic to the dams.

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Oral administration

DuPont Co. (1988). Unpublished Data, "Pilot Teratogenicity Study in Rats" (also cited in OTS05880617 and OTS0000617).

Merkle, J. and H. Zeller (1980). Arzneim.-Forsch., 30(9):1557-1562 (CA93:180514x).

Dermal administration

Stula, E. F. and W. C. Krauss (1977). Toxicol. Appl. Pharmacol., 41:35-55.

DuPont Co. (no date). Unpublished Data, "Embryotoxicity in Rats and Rabbits from Application of Chemicals to Skin During Organogenesis" (also cited in TSCA Fiche OTS0571481, OTS0571481, and OTS0206452).

Data from these additional sources were not summarized because insufficient information was available. These studies reported fetal effects; however, no information on maternal toxicity was reported.

Oral administration

DuPont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 117-65, "Evaluation of Monomethylformamide for possible Teratogenic and/or Abortifacient Properties" (August 13) (also cited in TSCA Fiche OTS0520896).

Oral, intraperitoneal, and dermal administration

Thiersch, J. B. (1971). Mason & Cie, Paris, 95-113.

Dermal administration

Stula, E. F. and W. C. Krauss (1977). Toxicol. Appl. Pharmacol., 41:35-55.

DuPont Co. (no date). Unpublished Data, "Embryotoxicity in Rats and Rabbits from Application of Chemicals to Skin During Organogenesis" (also cited in TSCA Fiche OTS0571481).

Shepard, T. H. (1986). Catalog of Teratogenic Agents, 5th ed., pp. 398, The John Hopkins University Press, Baltimore, Maryland (HSDB/100).

Gleich, J. (1974). Naunyn-Schmiedeberg's Arch. Pharmakol., 282 (Suppl.):R25 (HEEP/76/00574).

Subcutaneous administration

Welsh, J. J. (1979). Diss. Abstr. Int. B, 40(2):549 (CA 91:169465v) (RTECS No.

LQ3000000).

Intraperitoneal administration

Gleich, J. (1974). Naunyn-Schmiedeberg's Arch. Pharmakol., 282 (Suppl.):R25 (HEEP/76/00574).

Data from these additional sources were not summarized because insufficient information was available. These studies reported differential toxicity between the fetus and the mother; however, limited data were reported.

Oral administration

Thiersch, J. B. (1962). J. Reprod. Fertil., 81(4):219-220.

Oral and dermal administration

Roll, R. and F. Baer (1967). Arzniem.-Forsch., 17(5):610-614 (CA 67:52051v).

Intraperitoneal administration

Welsh, J. J. (1979). Diss. Abstr. Int. B, 40(2):549 (CA 91:169465v) (RTECS No. LQ3000000).

Kroeger, H. et al. (1984). Prog. Tryptophan Serotonin Res., Proc.-Meet. Int. Study Group Tryptophan Res. 4th, 685-688 (CA 101:67512r).

Kroeger, H. et al. (1983). Experientia, 39(l):93-94 (CA 98:66739c).

Oral, intraperitoneal, and dermal administration

Oettel, H. and G. Wilhelm (1957). Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol., 230:559-593 (CA 51:14993i).

Dermal administration

DuPont Co. (1967). Unpublished Data, Haskell Laboratory Report No. 88-67, "Embryonic Development Test" (June 12) (also cited in TSCA Fiche OTS0520898, OTS0571758, and OTS0571273).

DuPont Co. (1967). Unpublished Data, Haskell Laboratory Report No. 5-67, "The Effects of DMF, MMF, and Formamide on Embryonic Development in the Rat" (January 17) (also cited in TSCA Fiche OTS021530, OTS0520897, and OTS0546524).

Tuchmann-Duplessie, H. and L. Mercier-Parot (1965). C. R. Hebd. Seances Acad.

Sci., 261(l, Group 12):241-243.

Data from this additional source were not summarized because the result was inconsistent with the majority of the other findings.

Laitarenko, G. N. et al. (1992). Gig. Sanit., (2):30-32 (CA 118:2071).

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the study design was *in vitro* rather than *in vivo* and the data were not substantially additive to the database.

Groth, G. et al. (1990). Cah. Notes Doc., 140:712-715 (CA 114:76747s).

Data from these sources were not presented because the study design was *in vitro* and did not support existing *in vivo* data.

Klug, S. et al. (1998). Toxicol. *In Vitro*, 12(2):123-132 (CA 129:77796).

Karnofsky, D. A. (1955). Cancer Res., 15(Suppl. 3):83-85.

Karnofsky, D. A. and C. R. Lacon (1962). Cancer Res., 22(l, Part 2):84-86.

Supporting Data: Study No. 1

Species/Strain:	Rat/Sprague Dawley
Sex/Number:	Females/22-24 per group
Route of Administration:	Oral gavage
Exposure Period:	Gestation days 6-20
Exposure Levels:	0, 50, 100, 200, 300 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Time-mated rats were used on the study. Females had been housed overnight with adult males (1 male: 2-3 females) from the same strain). The day sperm was detected in the vaginal smear was considered to be gestation day 0. Mated females were housed singly.

The control group received the distilled water vehicle.

Females were observed daily for clinical signs of toxicity. Food consumption and body weights were periodically measured. Rats were killed on gestation day 21 and the uterus was removed and weighed. Uterine contents were examined to

determine the number of implantation sites, resorptions, and dead and live fetuses. Live fetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. Half of the live fetuses from each litter were preserved in Bouin's solution and examined for internal soft tissue changes via the techniques of Wilson and Barrow and Taylor. The other half of the fetuses in each litter were eviscerated and processed for skeletal examination.

The number of implantation sites and live fetuses and various body weights were analyzed by one-way analysis of variance, followed by Dunnett's test if differences were found. The frequency of resorptions and anomalies among litters was evaluated by using the Kruskal-Wallis test followed by the Dixon-Massey test where appropriate. Rates of pregnancy and fetal sex ratio were analyzed by using Fisher's test.

GLP: No data

Test Substance: DMF, purity 99.9%

Results: All females survived to scheduled termination. Maternal weight gain, maternal body weights, and food consumption were significantly reduced in the 100, 200, and 300 mg/kg groups.

Fetal body weight per litter was significantly reduced at 100 mg/kg and higher. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below.

	Dose (mg/kg/day)				
	0	50	100	200	300
% Pregnant	66.7	95.5	86.4	86.4	90.9
No. of litters examined	16	21	19	19	20
Mean implantation sites	15.81	14.48	15.47	15.53	15.25
Mean live fetuses	15.25	13.81	14.79	14.58	14.05
Mean % resorption sites	3.71	8.62	4.45	6.15	7.55
Fetal sex ratio (M/F)	1.05	0.91	0.90	1.08	0.92
Mean fetal body weight (g)	5.54 A 5.65 M 5.43 F	5.52 A 5.66 M 5.38 F	5.30 A 5.43 M 5.16 F	4.87 A 4.99 M 4.75 F	4.76 A 4.90 M 4.62 F
A = all fetuses; M = male; F = female					

Single occurrences of external and visceral malformations were observed in DMF groups. However, there was neither a specific pattern of malformations nor a significant increase in the incidence of total malformations. There were no significant

changes in the incidence of external or visceral variations. Statistically significant increases in the incidence of 2 skeletal variations, unossified or incompletely ossified supraoccipital and sternebrae, were seen in fetuses from the 200 and 300 mg/kg groups. A summary of fetal malformations and variations is provided in the table below. Incidences of findings are presented as number of fetuses (litters) affected.

	Dose (mg/kg/day)				
	0	50	100	200	300
Number of fetuses (litters) examined					
External exam	244 (16)	290 (20)	281 (19)	277 (19)	281 (20)
Visceral exam	122 (16)	145 (20)	141 (19)	138 (19)	141 (20)
Skeletal exam	122 (16)	145 (20)	140 (19)	139 (19)	140 (20)
Malformations					
Exophtalmia bilateral	0	0	0	0	1 (1)
Encephalocele	0	0	0	0	1 (1)
Agnatia	0	0	0	0	1 (1)
Absence of nasal septum	0	0	0	0	1 (1)
Interventricular septum defect	0	1 (1)	0	0	0
Diaphragmatic hernia	0	1 (1)	1 (1)	0	0
Hydronephrosis (bilateral)	0	0	0	1 (1)	1 (1)
Total number with malformations	**0**	**2 (2)**	**1 (1)**	**1 (1)**	**2 (2)**
External variations					
Hindlimb talipes	0	0	0	1 (1)	0
Rudimentary tail	0	0	1 (1)	0	0
Total number with external variations	**0**	**0**	**1 (1)**	**1 (1)**	**0**
Visceral variations					
Dilated renal pelvis	4 (2)	5 (5)	0	1 (1)	1 (1)
Dilated ureter	17 (8)	6 (4)	5 (5)	4 (4)	10 (4)
Total number with visceral variations	**17 (8)**	**10 (8)**	**5 (5)**	**5 (5)**	**11 (5)**
Skeletal variations					
Skull					
Parietals, incomplete ossification	2 (1)	0	0	0	0

Supraoccipital, imcomplete ossification (moderate)	0	1 (1)	8 (6)	52 (16)	49 (17)
Supraoccipital, absent or imcomplete ossification (Severe)	0	1 (1)	1 (1)	12 (9)	70 (16)
Total number with skull variations	2 (1)	2 (2)	9 (7)	64 (16)	119 (20)
Sternebrae					
5th absent or imcomplete ossification	3 (2)	12 (6)	13 (7)	15 (11)	32 (13)
2nd and 5th absent	0	1 (1)	0	0	0
Ribs					
13th short	0	0	0	0	1 (1)
Extra cervical	2 (2)	2 (2)	1 (1)	1 (1)	1 (1)
Extra lumbar	11 (7)	8 (4)	7 (7)	4 (3)	1 (1)
Vertebral centra, incomplete ossification	8 (7)	11 (7)	26 (11)	19 (10)	8 (4)
Total number with skeletal variations	**21 (11)**	**34 (13)**	**48 (16)**	**81 (19)**	**125 (20)**

DMF was not selectively toxic to the rat conceptus following oral administration. The NOAEL for maternal and developmental toxicity was 50 mg/kg/day. DMF was neither embryolethal nor teratogenic at doses up to 300 mg/kg/day.

Reference: Saillenfait, A. M. et al. (1997). Fund. Appl. Toxicol., 39:33-43.

Reliability: High because a scientifically defensible or guideline method was used.

Supporting Data: Study No. 2

Species/Strain: Rabbit/Chbb: HM (Russian)

Sex/Number: Females/11-24 rabbits per group

Route of Administration: Oral gavage

Exposure Period: Gestation days 6-18

Exposure Levels: 0, 46.4, 68.1, 200 µL/kg (ca. 44.1, 65, 190 mg/kg/day)

Method: No specific test guideline was reported; however, methods were according to FDA guidelines for reproduction studies for safety evaluation of drugs for human use.

The doses used for the study corresponded to ca. 1/45, 1/30, and 1/10 of the approximate 50% lethal dose. A total of 65 rabbits were used on the study with 24, 12, 18, and 11

rabbits used for the control, 44.1, 65, and 190 mg/kg/day groups, respectively. The rabbits were 20 to 47 weeks of age. The day of artificial insemination was designated as gestation day 0.

DMF was dissolved in aqua bidest and was administered at a dosage volume of 10 mL/kg. Clinical signs of toxicity, mortality, and food consumption were evaluated daily during the study. Body weights were recorded periodically throughout the study. The rabbits were sacrificed on gestation day 28 and were investigated by gross pathological examination. Uterine content was examined with respect to number of implantation sites, resorptions, number of live and dead fetuses, and the number of corpora lutea was counted. Sex, length, and weight of live fetuses and the respective placental weight was recorded. All fetuses were examined for external malformations, and for skeletal examination they were x-rayed in 2 levels (dorsoventral and lateral). The head of all fetuses were fixed in Bouin's solution and investigated according the to technique of Wilson.

GLP: No

Test Substance: DMF, purity not reported

Results: All animals survived until scheduled termination.

Maternal toxicity was evident in the 190 mg/kg group. Reduced body weights, body weight gain, and food consumption occurred in this dose group. Three dams aborted, 1 on day 21, 1 on day 24, and 1 on day 28. At necropsy, the liver of 1 dam was a clay-like color.

At 190 mg/kg, fertility index, number of corpora lutea, number of implantations, and the ratio of live/dead fetuses were unaffected. Placental weights and fetal weights, as well as fetal length were significantly decreased. The incidence of malformed fetuses observed in 7 litters was increased (16/45 = 35.5%). Hydrocephalus internus (6 fetuses), exophthalmia (2 fetuses), ectopia visceralis (3 fetuses), hernia umbilicalis (7 fetuses), and cleft palate (1 fetus) were observed. Three fetuses showed multiple malformations.

At 65 mg/kg, a transient reduction in maternal food consumption was observed during the treatment period; however, this had no effect on body weight or body weight gain. Gross necropsy revealed a clay-like colored liver in 1 dam.

At 65 mg/kg, the mean number of implantations and percentage of live fetuses was decreased; however, a dose-response relationship was not evident. Fetal parameters, number and type of variations and retardations were unchanged. Three malformed fetuses in 2 litters were found. This incidence was not statistically different from the control; however, the type of malformation (hydrocephalus internus) indicated a substance-related effect.

In the low-dose group (44.1 mg/kg), a transient reduction in maternal food consumption was observed during the treatment period without any effect on body weight or body weight gain. No substance-related pathological findings were recorded. Gestational and fetal parameters were unaffected. One malformed fetus (hydrocephalus internus) was found; however, this was in the range of the control.

The maternal NOEL was 65 mg/kg and the fetal NOEL was 44.1 mg/kg.

Reference: BASF AG (1976). Unpublished data (XXIII/402), December 14 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

Merkle, J. and H. Zeller (1980). Arzneimittel-Forsch. (Drug Res.), 30(9):1557-1562 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

Reliability: Medium because a suboptimal study design was used.

Supporting Data: Study No. 3

Species/Strain: Rat/Long Evans

Sex/Number: Females/22-23 per group

Route of Administration: Inhalation

Exposure Period: Gestation days 6-15

Frequency of treatment: 6 hours/day

Exposure Levels: 0, 18, 172 ppm

Method: No specific test guideline was reported; however, methods of Lorke, D. (1963). Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol., 246:147 and Lorke, D. (1965). Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol., 250:360 were used.

Female rats weighed 200-250 g and were 2.5-3.5 months old at

the beginning of the study. Two females were mated with one male overnight. Vaginal smears were prepared, and the day sperm were observed was designated as day 0 of gestation. At that time the females were housed singly.

DMF was dissolved in polyethylene glycol 400. Control animals inhaled 20 mm^3 polyethylene glycol/L air only. The DMF concentrations were determined by gas chromatography.

Cesarean sections were performed on gestation day 20. The average weight of fetuses/litter was determined. All fetuses were examined for external deformities. Approximately 1/3 of the fetuses were examined viscerally via the Wilson technique modified by Machemer and Stenger. The remaining fetuses were exenterated and abdominal and thoracal organs were evaluated. The fetuses were stained according to Dawson for skeletal examinations.

GLP: No

Test Substance: DMF, purity not reported

Results: The analytically measured concentrations were 17.8 and 172.3 ppm DMF for the 18 and 172 ppm groups, respectively.

All animals survived until scheduled cesarean section. The pregnancy rate was 85, 100, and 100% for the control, 18, and 172 ppm groups, respectively.

Fetal and maternal weights were reduced at 172 ppm. A summary of findings is presented in the table below.

	Concentration (ppm)		
	0	18	172
Maternal weight gain during gestation (g)	102.7	105.7	103.6
Maternal weight gain during treatment period (g)	36.3	36.9	33.5
No. of implantations	12.0	11.4	11.4
No. of fetuses	9.5	10.4	10.6
No. of resorptions	2.5	1.0	0.8
Avg. fetal weight (g)	4.07	4.03	3.78
Fetuses with slight bone changes	2.76	2.95	2.30
Fetuses with deformities	0.06	0.10	0.10

Stunted fetuses (<3 g)	0.06	0.20	0.20

The NOEL for maternal and developmental toxicity was 18 ppm.

Reference: Kimmerle, G. and L. Machemer (1975). Int. Arch. Arbeitsmedizin, 34:167-175 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

Reliability: Medium because a suboptimal study design was used.

Supporting Data: Study No. 4

Species/Strain: Rats/Crl:CD

Sex/Number: Females/21 per group

Route of Administration: Whole-body inhalation

Exposure Period: Gestation days 6-15

Frequency of treatment: 6 hours/day

Exposure Levels: 0, 30, 300 ppm

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Nulliparous female rats were 12-14 weeks of age upon arrival. Female rats were paired with male rats of the same strain 1:1, and mating was verified by the finding of sperm in the vaginal smear following overnight cohabitation. The day sperm was noted was designated as gestation day 0. Females were individually housed following mating.

Inhalation exposures were conducted in stainless steel and glass chambers (Rochester-type) with an effective volume of 760 L. DMF was generated by passing a stream of dry air through bubblers containing DMF. The air flow rate through the bubbler was monitored with a rotameter and was calibrated to volatilize the appropriate amount of test substance. The vapor-air mixture was diluted to the appropriate concentration with room air prior to entering the exposure chamber. Control rats were exposed to air only. DMF concentrations were measured by IR analysis.

Maternal body weights were recorded on gestation days 0, 6-15, and 21. Clinical signs were recorded daily. Cesarean sections took place on gestation day 21. Corpora lutea were counted, fetuses were removed from the uterus, and the number and position of all live, dead, and resorbed fetuses were

recorded. The fetuses were individually weighed, sexed, and examined for malformations. Approximately 2/3 of the fetuses from each litter were examined for visceral alterations. These fetuses were then eviscerated and stained for skeletal examinations. Approximately 1/3 of the fetuses were fixed in Bouin's solution and examined for neural and visceral defects using the technique of Wilson.

Comparisons between control and DMF-treated groups were made, where applicable, by the chi-square method. Body weights, body weight gains, numbers of corpora lutea, implantations of the dams, number of fetuses per sex, fetal and litter weights, crown-rump distances, and the number of ossification variations/fetus/litter were compared to control by the F-test and Student's t-test. When variances differed significantly, Student's t-test was appropriately modified using Cochran's approximation. Mean reproduction data were compared to control by the one-tailed t-test.

GLP: No data

Test Substance: DMF, purity not reported

Results: The mean exposure concentrations for this study were 0, 31.2, and 297 ppm for the 0, 30, and 300 ppm groups, respectively.

No mortality was observed during the study. No adverse clinical signs were noted in DMF-exposed rats. Maternal weight gains were reduced in the 300 ppm group. There were no test substance-related necropsy findings.

A summary of reproductive outcomes is provided in the table below. Reductions in the numbers of implantations, fetuses, and corpora lutea were observed in the 30 ppm group. Since these findings were not observed in the 300 ppm group, they were not considered to be DMF-related findings.

	DMF Concentration (ppm)		
	0	30	300
% Pregnant	100	100	100
Corpora lutea/female	15.3	14.6	14.4
Implantations/ female	14.2	12.7	14.4
Resorptions/ female	0.5	0.8	0.5
Live fetuses/female	13.7	12.0	13.9
Mean fetal weight (g)	5.5	5.5	5.3

Viable fetuses/ implantation site (%)	96.5	94.5	96.5
Mean crown-to-rump (cm)	4.0	4.0	4.0
Sex ratio	NR	NR	NR
NR = Not reported			

Soft tissue malformations indicated a high incidence with distended renal pelvis and/or ureters in all groups. The total incidence of fetuses and litters containing fetuses with soft tissue malformations were comparable between the control group and the DMF-treated groups. Skeletal malformations were not unusual and included angulated, wavy, or extra ribs, fused thoracic vertebrae, split sternebrae, and misshapen scapula. These findings were distributed throughout the groups, were low in incidence, and do not suggest any relationship to DMF exposure. A summary of fetal malformations and variations is provided in the table below. Incidences of findings are presented as number of fetuses affected. The number in parenthesis represents percent of those examined.

	DMF Concentration (ppm)		
	0	30	300
No. examined externally	288	251	291
Diaphragmatic hernia	0 (0)	1 (0.4)	0 (0)
Lens-vacuoles	0 (0)	1 (0.4)	2 (0.7)
No. examined viscerally	97	81	99
Distended renal pelvis	31 (32.0)	17 (21.0)	26 (26.3)
Distended renal pelvis and ureter	6 (6.2)	4 (4.9)	7 (7.1)
Distended renal pelvis, ureter, and bladder	1 (1.0)	0 (0.0)	0 (0.0)
Distended cerebral ventricles	1 (1.0)	0 (0.0)	0 (0.0)
No. examined skeletally	191	170	192
Misshapen scapula	1 (0.5)	0 (0.0)	0 (0.0)
Angulated ribs	1 (0.5)	2 (1.2)	0 (0.0)
Wavy ribs	0 (0.0)	1 (0.6)	0 (0.0)
Cervical rib	0 (0.0)	1 (0.6)	0 (0.0)

Fused thoracic vertebrae	0 (0.0)	1 (0.6)	0 (0.0)
Split sternebrae	0 (0.0)	0 (0.0)	1 (0.5)

Maternal and fetal toxicity were evident at 300 ppm as evidenced by reduced maternal and fetal weight. The NOEL for both maternal and fetal toxicity was 30 ppm.

Reference: Lewis, S. C. et al. (1992). Drug. Chem. Toxicol., 15(1):1-14.

Reliability: Medium because a suboptimal study design was used.

5.4 Reproductive Toxicity

Study No. 1

Species/Strain: Mice/Swiss
Rats/Wistar AG

Sex/Number: Male and female/20 per group

Route of Administration: Intraperitoneal injection

Exposure Period: See methods section

Frequency of Treatment: See methods section

Exposure Levels: See methods section

Method: No specific test guideline was reported. An acute lethality study was conducted as well as a repeated dose study. MMF was dissolved in isotonic saline solution (50 g/100 mL).

In the acute study, mortality was recorded. Clinical signs were recorded periodically. Animals which died as a consequence of administration, as well as the survivors which were sacrificed 30 days after administration, were subjected to necropsy. Ten organs, including the testicles, were removed for pathoanatomic examination.

In the repeated dose study, 20 male rats received 1/20 of the sublethal dose. An additional group of 20 male rats received isotonic saline, and another 20 male rats received no treatment. Rats received 5 injections per week for approximately 7 weeks. The rats received a total of 36 injections. Clinical signs and body weight were recorded. After 15 injections, 5 rats/group and after the 36th injection, all remaining rats were placed in metabolism cages. Urine was collected after 24 hours in the cages. After the 24-hour period, the rats were sacrificed. Blood was taken for hematological evaluation. Pathoanatomic evaluation was conducted on 10 organs, including the testicles.

GLP: No

Test Substance: N-methylformamide, purity not reported

Results: In the acute study, an adverse effect on the seminiferous tubules was observed in rats and mice treated with MMF. The germinal epithelium was detached from the vitreous hyaline and numerous spermatogonia and spermatocytes were necrosed. These testicular lesions were not observed in the survivors sacrificed 30 days after the administration of MMF.

In the repeated dose study, pathoanatomic examination revealed no microscopically demonstrable cellular lesions.

The authors state that their results confirm the findings of Lechat, P. et al. (1960). C. R. Acad. Sci. Paris, 251:1937-1939 and Wallon, D. et al. (1960). Le Sang, 31(9):871-879. In these studies, the authors reported that the testicular lesions were the result of karyoclastic activity which was also manifested in the bone marrow, ileum, and liver.

Reference: Chanh, P.-H. et al. (1971). Therapie, 26(3):409-424.

Reliability: Not assignable because limited study information was available.

Additional Reference for Reproductive Toxicity:

Data from this additional source were not summarized because insufficient study information available.

Mittler, S., J. (1961). J. Heredity, 52:163-166 (CA 56:5361g).

Supporting Data: Study No. 1 (One-generation study)

Species/Strain: Rats/CD

Sex/Number: Male and female/10 male and 20 female per group

Route of Administration: Dermal

Exposure Period: Up to 164 days

Frequency of Treatment: Daily

Exposure Levels: 0, 500, 1000, 2000 mg/kg/day

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Three treatment sequences were employed during the experiment as follows:
Sequence 1: male and female rats were treated with 500, 1000, or 2000 mg/kg DMF during test day 0 through 28

(pre-mating period), and with distilled water for the rest of the study.
Sequence 2: female rats were treated with 500, 1000, or 2000 mg/kg DMF during both mating and gestation periods, and with distilled water during pre-mating and lactation periods. Males received distilled water throughout the entire investigation.
Sequence 3: male and female rats were treated with 500, 1000, or 2000 mg/kg throughout the entire investigation (premating, mating, gestation, and lactation periods).
Two control groups (distilled water) were also used in the study.

Premating occurred during test days 0-28 and again during test days 29-56. The first mating occurred during test days 57-72, the first gestation was during test days 73-94, the first lactation was during test days 95-116. A rest period occurred during test days 117-127. Mating occurred again during test days 128-143 followed by the second gestation during test days 144-164.

DMF was applied during the dosage periods to the skin on the backs of the animals. The skin had been previously clipped of hair.

Body weights, clinical signs, and mortality data were collected periodically during the study. Parental animals were also evaluated for fertility, length of gestation, and lactation performance.

Mating trials were initiated when the rats were 100 days old (56 days on test). Females were caged in pairs and mated with a male from within the same treatment group.

The first litters obtained (F1a) were weaned 21 days post-partum. Pups were examined for physical abnormalities at birth and again at weaning. Records of live birth and pup survival at various stages of the lactation period were maintained.

Parental animals were then given a 10-day rest period and then mated again. All females were sacrificed on the 20th day of the second (F1b) gestation period. Uterine contents were examined. Fetal swellings and implantation sites were counted, with special attention being paid to resorption sites or uterine abnormalities. The number of corpora lutea were

also counted. Each fetus was given an external examination. All fetuses from the control and 2000 mg/kg DMF groups were examined for either skeletal or internal development. Where possible, equal number of fetuses of each sex from each litter were examined by each method. Evaluation of skeletal development was conducted by the Hurley's method. Internal development was evaluated using the free-hand razor blade section technique of Wilson and Warkany.

Males and females which failed to become pregnant were sacrificed at the conclusion of the second breeding period. Females that conceived the F1b litter were sacrificed on the 20th day of gestation period. A complete gross pathological examination was performed on all sacrificed animals and samples of liver, kidney, and gonadal tissues from 10 male and 10 females of each group were preserved. Microscopic examination of these tissues was conducted for 10 males and 10 females of the control and high-dose group or each sequence.

GLP: No

Test Substance: DMF, purity not reported

Results: Body weight gains were reduced in the 1000 and 2000 mg/kg males during the pre-mating treatment periods (sequences 1 and 3). The final body weights of males receiving 1000 or 2000 mg/kg (sequence 3) were less than the control males. Females treated with 2000 mg/kg during sequence 3 gained less weight than control females during the pre-mating phase. The final body weights of 2000 mg/kg females were less than the control females during both sequences 2 and 3.

An increase in mortality occurred in the 1000 and 2000 mg/kg groups during dosage sequence 3. Lung consolidation was noted during the gross pathology exam and death was attributed to chronic respiratory infection.

There were no untoward behavioral observations noted among parental animals during the investigation. Gross and histopathologic examination of the control and high-dose animals revealed no differences between control and test groups.

No differences between the control and test groups were noted in the parameters assessed for reproductive ability.

Fewer pups were delivered and retained during the lactation

period by females given 2000 mg/kg during sequences 2 and 3. Pup survival indices, calculated at various intervals during lactation, revealed a reduction of survival for pups delivered by dams given 1000 or 2000 mg/kg during sequences 2 and 3. Weanling body weights for the test groups were similar to the controls.

For the F1b litters, administration of 2000 mg/kg in sequences 1, 2, and 3 resulted in a reduction in the number of viable pups per litter. These decreases appeared to be the result of reduced numbers of corpora lutea and implantation sites per female in those groups. Treatment with 500 or 1000 mg/kg did not affect ovulation, implantation, or fetal survival. Fetal body weights were not affected by prenatal exposure to DMF. No differences between control and treated groups were noted in the fetal external examination. An increase in the percent of fetuses with incompletely or non-ossified sternum sections was observed among pups from the 2000 mg/kg groups. These values were within the expected range for the testing laboratory and were therefore not attributed to the administration of DMF.

Reference: Industrial Bio-Test Laboratories, Inc. (1973). Report No. IBT B161-B, "One-Generation Reproduction and Teratology Study with DMF in Albino Rats" (March 27) (TSCA Fiche OTS0518158).

Reliability: High because a scientifically defensible or guideline method was used.

Supporting Data: Study No. 2 (Continuous Breeding Study)

Species/Strain: Mouse/CD-1®(ICR)BR

Sex/Number: Male and female/see methods section

Route of Administration: Drinking water

Exposure Period: See method section

Frequency of Treatment: Continuously in the drinking water

Exposure Levels: 0, 1000, 4000, 7000 ppm (ca. 219, 820, and 1455 mg/kg/day)

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The RACB protocol was used and consisted of 4 segments: 1) a dose range-finding phase, 2) an F0 cohabitation and lactation phase, 3) a crossover mating trial of the F0 generation (conducted if F0 reproductive performance was

affected), and 4) a fertility assessment of the F1 generation (born and reared during the F0 lactation phase).

Mice were 6 weeks old upon arrival.

Range-finding study:
Dose levels used in the range-finding study were 0, 2500, 5000, 7500, 10,000, and 15,000 ppm in deionized, filtered drinking water. Male and females (8 per sex per group), 8 weeks of age, were used in the range-finding study. Food and water consumption and body weights were measured weekly during the 2-week exposure period. At the end of the 2 weeks, the animals were sacrificed with no further data collection.

F0 cohabitation and lactation study:
Male and female mice, 11 weeks of age, were assigned to 1 of 4 dose groups. The control groups consisted of 40 males and 40 females, and the test groups consisted of 20 males and 20 females. Doses used for this study were 0, 1000, 4000, and 7000 ppm. Body weights and food and water consumption were monitored periodically throughout the study. During week 1 of this study, the animals were individually housed. During weeks 2-15, mice were housed in breeding pairs within dose groups. Newborn litters were sacrificed immediately after evaluation. Data collected included litter interval, number, sex, weight of pups per litter, number of litters per breeding pair, and the postnatal day (PND) 0 body weight. The breeding pairs were separated at week 16 and F0 females were allowed to deliver and rear the final litter until PND 21. Pups were sexed, counted, and weighed periodically through the postnatal phase. On PND 21, randomly selected F1 pups from each dose group were weaned and housed in same-sex pairs by dose and saved for the F1 fertility assessment phase (described below). After completion of the cohabitation and lactation phase, all F0 animals were maintained on their respective treatment until scheduled sacrifice after the completion of the crossover mating phase.

Crossover mating trial (conducted because fertility was affected in the F0 cohabitation and lactation study):
This study was conducted using the control and high-dose mice. Three breeding groups of F0 animals were created: 1) control male x control female, 2) high-dose male x control female, and 3) control male x high-dose female. Beginning

at week 23 of treatment, mice were cohabited until a vaginal copulatory plug was observed or for 1 week. No treatments were administered during cohabitation. Mice were singly housed at week 24 and dosing resumed. Upon delivery of each litter, lethality, gestation length, sex, number, weight of pups, and dam weight were recorded. All newborn litters were sacrificed following evaluation. After all litters had been delivered, vaginal smears were recorded for 12 days. At week 29, all F0 male and female mice were weighed, sacrificed, and necropsied. Liver and paired kidney weights were recorded for both sexes. Right testis, right epididymis, prostate, and seminal vesicles with coagulating glands were also weighed. The right ovary with attached oviducts was weighed in females. All tissues were fixed and embedded in paraffin. Sperm evaluations from the right testis included manual assessments of motility, concentration, and morphology. Homogenization-resistant spermatid head were counted from the left testis. Histopathologic evaluations were conducted on all livers, right and left kidneys and adrenals, the right testis and epididymis, prostate, seminal vesicles, ovary, and any gross lesions noted during necropsy.

F1 fertility assessment:
At weaning (PND 21), randomly selected F1 pups from the control, 1000, 4000, and 7000 ppm groups were housed 2 per cage by sex within dose group. On PND 22, the pups began directly receiving DMF via the drinking water. At 74±10 days of age, males and females in the control or treated groups were cohabited as nonsibling breeding pairs until a vaginal copulatory plug was observed or for 1 week. Although 20 non-sibling pairs was the goal, reduced survival in the high-dose group, allowed only for 15 pairs, some of which were siblings. Litter data were collected as described above for the F0 adults in the crossover mating study. After delivery of the F2 litters, vaginal smears were collected for 12 days for the F1 females. Body weight and food and water consumption were recorded periodically. At necropsy, F1 males and females were weighed and data collected as described above for the F0 animals. Histopathological evaluations were conducted on all livers, right and left kidneys and adrenals, the right testis and epididymis, ovary, and any gross lesions noted during necropsy.

Selected F2 litters were preserved on PND 1 and evaluated for whole body skeletal malformations and soft tissue malformations of the head. Selected adult F1 males and

females were evaluated for skeletal malformations.

Statistical analysis:
Most hypotheses were tested using the Williams' modification on Dunn's or Shirley's nonparametric multiple comparison procedures. Jonckheere's test was used to ascertain whether there was sufficient evidence of a dose-related response to apply Shirley's test. For data expressed as a proportion, the Cochran-Armitage test was used to test for a dose-related trend, and pairwise comparisons were performed using a Chi-square test.

GLP: Yes

Test Substance: DMF, purity > 99%

Results: Range-finding study:
Treatment-related deaths occurred at doses of 10,000 (3 males) and 15,000 ppm (7 males and 3 females). Body weight was decreased in the remaining 15,000 ppm mice. Water consumption was decreased in both sexes at weeks 1 and 2. Doses of 1000, 4000, and 7000 ppm were chosen for the continuous breeding study.

Cohabitation and lactation studies (continuous breeding phase):
For F0 animals, there was no increased incidence of mortality and no dose-related clinical signs of toxicity. There was no effect on male body weight, food consumption or water consumption. Female body weight was significantly reduced at 7000 ppm at weeks 8 and 16, reflecting at least in part the non-pregnant status in 20-40% of the animals. For those mice that delivered a litter, body weight was affected by treatment at all doses by week 16.

During the lactational period, relative maternal feed consumption was significantly depressed at 7000 ppm on PND 0-4, at = 4000 ppm midlactation, and at = 1000 ppm on PND14-21. Relative maternal water consumption exhibited a similar, but more pronounced, effect.

At 7000 ppm, fertility was reduced in the first litter to 90%, compared to 100% in the controls. Over time, this treatment-related effect increased. By the final litter, fertility was reduced to 55% at 7000 ppm and reduced fertility was also noted at 4000 ppm. The average number of litters per pair, average litter size, proportion of pups born alive, and average pup weight were all reduced compared to control pairs. There was no effect on these parameters in the

1000 ppm group. A summary of reproductive outcomes is provided in the table below.

	Concentration (ppm)			
	0	1000	4000	7000
Number of breeding pairs	38	20	20	20
Percent fertile (1st litter)	100	100	100	90
Percent fertile (final litter)	92	95	70	55
Cummulative days to litter (1st litter)	21.7	24.5	28.1	23.1
Cummulative days to litter (final litter)	103	105	104	104
Litters per pair	4.9	4.8	4.5	3.8
Live pups per litter	11.8	11.8	7.5	5.3
Percent of live pups	98	99	76	71
Live pup weight (g)	1.58	1.55	1.30	1.27
Adjusted live pup weight	1.59	1.55	1.30	1.26

Pups born to DMF-treated pairs had external malformations and other abnormalities, including domed head and hematomas along the nose and on the head. Those pups most severely affected died shortly after birth, and many were cannibalized prior to examination. The proportion of litters with one or more pups with an abnormal appearance was 7.9, 10.5, 90.0, and 77.8% for the 0, 1000, 4000, and 7000 ppm groups, respectively. The reduction in the proportions of litters with malformed pups in the high-dose group, compared to the mid-dose group, was influenced by the decreased fertility, increased prenatal death, and postnatal cannibalism observed in the high dose group.

During the lactation phase, average post-natal survival was reduced in the 4000 and 7000 ppm groups. Live pup weight, reduced at birth, was affected only infrequently during the preweaning period.

Crossover mating trial and F0 necropsy:

No differences were detected in DMF-treated groups of either sex for comparisons with controls. The control group had a lower than usual pregnancy rate which resulted in fewer control litters, thus affecting the power of statistical analyses and the strength of the conclusions. Although no differences were observed between the treated groups and controls, differences between the treated groups were noted. Females treated with 7000 ppm (and mated with control males) produced fewer live pups per litter (5.5±1.0 vs. 10.2±1.2) when compared to the males treated with 7000 ppm (and mated with control females). Pup weights were also lower in the pups of dosed females compared to those sired by treated males. These data suggest that the female was the sex affected by DMF exposure. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below.

	Exposure Group		
	Control male x control female	7000 ppm male x control female	Control male x 7000 ppm female
Percent fertility	50 (8/16)a	69 (11/16)	55 (11/20)
Live pups per litter	8.1 (8)b	10.2 (11)	5.5 (11)
Live pup weight (g)	1.56 (6)c	1.63 (11)	1.44 (10)
Proportion of pups born alive	0.73	0.94	0.68
Adjusted live pup weight (g)	1.61d	1.66	1.38
Average dam weight (g)	40.30	41.42	40.74
Average days to litter	21.6	22.0	21.6

a = number of deliveries/number cohabited
b = numbers in parentheses are the number of dams delivering litters
c = numbers in parentheses are the number of litters with live pups
d = body weight was adjusted statistically to account for differences in litter size

Pups born to the treated females exhibited the same spectrum of malformations as observed during the continuous breeding phase (see the table below). Incomplete ossification of the cranial bones accounted for 82% of the malformations observed in the control male x control female group, and 97% of the malformations in the 7000 ppm male x control female group, but was not observed in the control male x 7000 ppm female group. Malformations observed in the control male x 7000 ppm female group included

abnormal ossification of the cranial plates, abnormal suture formation in the cranium, and abnormal or incomplete formation of the sternebrae. Examination of 95 heads from randomly selected pups revealed that 23.1% of the pups born to DMF-treated mothers had malformations, including agenesis of the cerebrum, agnathia, abnormally shaped centrum or cranium, cleft palate, or enlarged cerebral ventricles. Head malformations from the other 2 groups were accounted for solely by the finding of enlarged nasal passages.

	Exposure Group		
	Control male x control female	7000 ppm male x control female	Control male x 7000 ppm female
Proportion of litters with 1 or more externally malformed pups	12.5%	0.0%	90.9%
Proportion of litters with 1 or more internally malformed pups	83.3%	81.8%	100%
Percent of pups (within litters) with skeletal malformations	40%	38%	95%

There was no effect on the length of the estrous cycle or stage frequency distribution; however, 86% of the controls had 4- or 5-day estrous cycles, compared to 66% after DMF exposure. Thus, the number of animals having normal cycles was affected by DMF.

At necropsy, DMF-treated F0 females had significantly depressed body weights. Male body weights were not affected. Male liver weights were increased at all doses. Female absolute and relative liver weight and kidney plus adrenal weights were increased at all doses. Histopathologic examination of animals with gross liver lesions (2 mid-dose females and 2 high-dose males) revealed centrilobular hepatocellular hypertrophy, which was considered treatment-related. Caudal epididymidal weight was significantly increased at all doses of DMF. A slight decrease in testicular spermatid concentration in the DMF-treated animals was significant in the low and high doses, with a significant trend present. DMF had no adverse effect on epididymal spermatozoan concentration, motility, or morphology. Histopathologic examination of the

reproductive organs found no findings related to DMF treatment. Therefore, the authors conclude that the effect on testicular spermatids was likely a Type II error and not biologically relevant.

Growth and survival of F1 juveniles:
The proportion of F1 pups born alive in the final litter and postnatal survival on postnatal day (PND) 4 were reduced in the mid- and high-dose groups and continued to decline throughout the lactation period. Pup weight during lactation was reduced in the mid- and high-dose groups prior to PND7 and may have contributed to decreased survival rate. The F1 pups in the mid- and high-dose groups also exhibited craniofacial malformations. Pups that were severely malformed did not survive the preweaning period. The surviving F1 pups were closely examined and those in the mid- and high-dose groups were small and appeared to have foreshortened, domed heads.

After weaning, pups were randomly selected for rearing and inclusion in the reproductive performance evaluation of the F1 generation. Both male and female body weights were reduced in the mid- and high-dose groups throughout the remainder of the study. Food consumption was unaffected in the F1 generation. Water consumption was increased for the males in the mid- and high-dose groups. Estimated mean exposure to DMF was 259, 1023, and 1934 mg/kg/day for the 1000, 4000, and 7000 ppm groups, respectively, with females receiving slightly more DMF than males.

Reproductive performance of the second generation:
The mating index was significantly decreased at 7000 ppm. Fertility was reduced in the 4000 and 7000 ppm groups. The average days to litter was increased, and the number of live pups per litter, pup body weight, and the proportion of pups born alive was decreased in the 4000 and 7000 ppm groups. Live pup weight was also decreased in the 1000 ppm pups. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below.

	Concentration (ppm)			
	0	1000	4000	7000
Percent fertile	90 (18/20)	90 (18/20)	56 (10/18)	53 (8/15)
Live F2 pups per litter	11.3	11.8	4.9	4.1

Proportion of pups born alive	1.00	0.99	0.74	0.56
Live F2 pup weight (g)	1.59	1.48	1.30	1.32
Adjusted live F2 pup weight (g)	1.61	1.52	1.21	1.23
Average dam weight (g)	34.9	34.7	30.2	28.9
Average days to litter	21.2	21.6	23.0	23.5

F2 pups born to DMF-treated F1 pairs exhibited malformations similar to those observed for the F1 litters of F0 pairs. The proportion of litters with 1 or more externally malformed pups was 0, 27.7, 60, and 75% for the 0, 1000, 4000, and 7000 ppm groups, respectively.

F1 estrous cycles were monitored after the birth of the F2 pups. Females in the 7000 ppm group had significantly longer cycles and tended to be in either metestrus or diestrus longer than control animals.

At necropsy, F1 male and female body weights were reduced in the 4000 and 7000 ppm groups. Absolute and relative liver weights were significantly increased in all DMF-treated animals of both sexes. Female relative kidney plus adrenal weight was increased in the 4000 and 7000 ppm groups. Histopathological examination of the animals (in low and high dose groups) with gross lesions revealed treatment-related centrilobular hepatocellular hypertrophy. These finding indicated a general toxicity at = 1000 ppm.

Evaluation of F1 reproductive tissues revealed some significant effects in males but not females. Relative prostate weight was decreased at all doses, as was absolute prostate weight in males in the 4000 and 7000 ppm doses. Epididymidal spermatozoa concentrations was decreased at 7000 ppm, but no other significant effects of treatment were noted for andrologic parameters. Relative ovary weight was increased in the 4000 ppm females due to the presence of cystic ovaries in 2 animals, but was not considered treatment-related.

Developmental effects observed at delivery were confirmed in surviving F1 animals. Malformations observed in the

mid- and high-dose animals consisted of abnormal or incomplete ossification of the cranial plates, abnormal cranial suture formation, and abnormally formed sternebrae. Histopathologic evaluation of additional F1 animals in the mid- and high-dose group revealed dysplasia of the cranial bones, primarily at the midline.

Significant reproductive and developmental toxicity was observed in both generations at 4000 and 7000 ppm DMF in the presence of some general toxicity. The liver appeared to be the primary non-reproductive target organ. Reduced F2 pup weight was noted at 1000 ppm DMF.

The NOEL for the parental generation was < 1000 ppm. The NOEL for the F1 offspring was 1000 ppm and the NOEL for the F2 offspring was < 1000 ppm.

Reference: Fail, P. A. et al. (1998). Reprod. Toxicol., 12(3):317-332.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Reproductive Toxicity: None Found.

5.5 Genetic Toxicity

Type: ***In vitro* Bacterial Mutations**

Tester Strain: *Escherichia coli*, strains Sd-4, Sd-4-73, WP-14, WP-2

Exogenous Metabolic Activation: None

Exposure Concentrations: 10, 20, 50 mg/mL

Method: No specific test guideline was reported.

The test material was dissolved in sterile distilled water. The bacteria were grown for 24 hours at 37ºC in an aerated broth culture. The streptomycin-dependent strains were supplemented with 10 µg of streptomycin per mL culture media. Five mL samples of fully grown culture were centrifuged, washed in saline, and resuspended in distilled water or buffer. These suspensions were kept for 1 to 3 hours at 37ºC. After treatment, suspensions showing microscopically detectable clumping were not used in the experiment.

The numbers of viable bacteria in the control and treated suspensions were assayed by plating samples of suitable

	dilutions on nutrient broth agar, supplemented with streptomycin if applicable. In the auxotrophic strains, the number of revertants to nondeficiency was determined by plating samples of the suspension on synthetic medium enriched with dehydrated nutrient broth. The streptomycin-dependent strains were plated on nutrient broth agar in order to determine the number of reversions to nondependence. Reversions of cys-2 in strain Sd-4-73 were scored by plating on enriched synthetic medium to which streptomycin had been added. The frequency of observed mutations was obtained by dividing the number of mutant colonies, corrected for the estimated number of spontaneously occurring mutants, by the number of viable bacteria plated, and recalculating on the basis of 10^8 viable cells.
GLP:	No
Test Substance:	N-methylformamide, purity not reported.
Results:	Negative
Remarks:	No additional data.
Reference:	Hemmerly, J. and M. Demerec (1955). Cancer Res., 15(Suppl. 3):69-75.
Reliability:	Low because an inappropriate method or study design was used.

Additional References for *In vitro* Bacterial Reverse Mutation Assay:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Laitarenko, G. N. et al. (1992). Gig. Sanit., (2):30-32 (CA 118:2071).

Arundel, C. M. and P. J. Tofilon (1987). Radiat. Res., 110(2):294-304 (TOXBIB/87/204951).

Supporting Data: DMF

Type:	***In vitro* Bacterial Reverse Mutation Assay**
Tester Strain:	*Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S9
Exposure Concentrations:	0, 0.94×10^4, 2.4×10^4, 4.7×10^4, 9.4×10^4, 19×10^4, 47×10^4 µg/plate

Method: No specific test guideline was reported; however, procedures generally followed those outlined in Ames et al. (1975). Mutat. Res., 31:347-364.

In the absence of an activation system, a solution of the test sample and approximately 10^8 bacteria were added to top agar. These components were mixed and poured on the surface of a plate containing Davis minimal agar. To treat in the presence of an activation system, S9 mix was added to the bacteria-test sample-top agar mixture. The S9 mix contained S9, $MgCl_2$, KCl, glucose-6-phospahte, NADP, and sodium phosphate. Once the S9 was added to the test sample and top agar, the components were mixed and immediately poured over the minimal agar plate.

The revertant colonies were counted after the plates were incubated at 37ºC for 48 hours.

Positive controls (2-aminoanthracene (2-AA), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 9-aminoacridine (9AAc), and 2-nitrofluorene (2NF)) and negative controls (solvent) were included in each assay.

The cytotoxicity of the test sample in the presence and absence of an activation system, as measured in strain TA1535, was the basis for selecting concentrations to be used in the mutagenesis experiment. The protocol used to determine cytotoxicity was identical to the mutagenesis protocol except that 10^3 rather than 10^8 bacteria were used per plate and a non-limiting concentration of histidine was present.

Data from replicate plates within a single experiment were averaged. The average of these values from different experiments was determined. The highest average number of revertants that was obtained was expressed as a multiple of the control value for the sensitive strain(s). When a test sample was active, the average numbers of revertants observed before activity plateaus or decreases at the various concentrations tested were submitted to linear regression analysis. The slope of the line thus obtained was used to determine the number of revertants/nmole or µg of test sample.

GLP: No

Test Substance: Dimethylformamide, purity 100%

Results: Negative

Remarks: The cytotoxicity data for DMF is provided in the table below.

S9 activation	Concentration (µg/plate)	% of Control Survival
-	$47x10^4$	<0.2
-	$19x10^4$	70
+	$47x10^4$	20
+	$19x10^4$	50

Normally, concentrations of test sample that give less than 50% of control survival are not selected for the mutagenesis assay. However, since these experiments were initiated to aid in evaluating a Utah Biological Testing Service (UBTS) Report, 500 µg/plate was chosen as the highest concentration.

A previous study conducted by DuPont (DuPont Co. (1976). Unpublished data, Haskell Laboratory Report No. HL 424-76) indicated that DMF tested at concentrations = 10 mg/plate were not mutagenic in the Ames test. However, the UBTS reported that DMF was mutagenic (tested at concentrations greater than 10 mg/plate). This data prompted DuPont to conduct the present test. Data from this test indicated that DMF was not mutagenic, even at concentrations that are cytotoxic. However, by testing a toxic concentration of DMF, a possible technical explanation for the conclusions of the UBTS study became apparent. While the number of revertants per plate at all concentrations of DMF never rose above the spontaneous frequency, a substantial number (about 1000) of small colonies (mutants, not revertants) was present in dishes that received 500 µL of DMF per plate. This effect was observed in all strains. The dishes that contained these small colonies did not possess the background lawn of mutants that are present under conditions of high survival. The presence or absence of a background lawn was confirmed by microscopic observation. The absence of a background lawn is indicative of toxicity.

Colonies from TA98 and TA1535 that received 500 µL and 100 µL of DMF were selected and streaked out on fresh plates that did or did not contain histidine. The colonies from the 100 µg plates were counted as real revertants. A survivor of toxicity (a mutant) should be able to grow in the presence of histidine, but not its absence, while a revertant show grow under both conditions. None of the colonies from the 500 µL plate grew in the absence of histidine while

all grew in its presence. Since biotin was not present in the -his plates and the colonies plated under the 2 conditions were not identical, each colony that grew on the complete plate was restreaked on –his, +his, and complete plates. The results confirmed that the colonies were composed of mutants, not revertants. Colonies (5/5) that were considered to be revertants from TA98 and TA1535 that received 100 µL of DMF grew on –his plates.

These data clearly show that the colonies that appeared when highly toxic concentrations were used, were survivors from the parent mutant population, not revertants to histidine in dependence. It appears that theses surviving mutants were counted as revertants in the UBTS study, leading to their conclusion that DMF was mutagenic.

Reference: DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. HL 818-77 (October 14).

Reliability: High because a scientifically defensible or guideline method was used.

Data from similar assays reported in these additional sources support the "negative" study results summarized above.

Antoine, J. L. et al. (1983). Toxicology, 26(3-4):207-212.

Baker, R. S. U. and A. M. Bonin (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program): 249-260 (CA95:216181).

Brams, A. et al. (1987). Toxicol. Lett., 38:123-133.

Brooks, T. M. and B. J. Dean (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.,: Rep. Int. Collab. Program):261-270 (CA95:216182).

Commoner, B. (1976). Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Noncarcinogenic Chemicals, EPA Pub. No. EPA-600/1-76-022, NTIS Pub. No. PB 259934 (NTIS).

de Serres, G. S. and S. Ashby (1981). Evaluation of Short-Term Tests for Carcinogens Progress in Mutation Research, Bd. 1, originally cited in BUA-Stoffdossier, N, N-dimethylformamid, Stand 04/91 (cited in IUCLID (1995). IUCLID Data Sheet, "N,N-dimethylformamide" (October 23)).

Dorado, G. and C. Pueyo (1988). Cancer Res., 48:907-912.

Dow Corning Corp. (1979). Unpublished Data, "Mutagenicity Evaluation of Dimethyl Formamide in the Ames Bacterial Assay," (Janaury 3) (TSCA Fiche OTS0520464).

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. HL-424-76, "*In Vitro* Microbial Mutagenicity Studies of DMF" (June 11).

Falck, K. et al. (1985). Mutat. Res., 150:119-125 (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

Green, N. R. and J. R. Savage (1978). Mutat. Res., 57:115-121.

Ichinotsubo, D. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):195-198.

Ichinotsubo, D. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):298-301 (CA98:192793d).

Imperial Chemical Industries (ICI) (1976). TSCA Fiche OTS0520485 (08-01-76) (cited in SIDS Dossier (2003). "Dimethylformamide" May 28 (draft) accessed from OECD website on Oct. 29, 2003).

Kada, T. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):175-182.

Karamova, L. M. and A. T. Shakirova (1990). Gig. Tr. Prof. Zabol., (4):54-55 (CA114:11459e).

Kassinova, G. V. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):434-455.

Loprieno, N. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):424-433.

MacDonald, D. J. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):285-297.

Matsushima, T. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):387-395.

Mehta, R. D. and R. C. Von Borstel (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):414-423.

Mohn, G. R. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):396-413.

Mortelmans, K. et al. (1986). Environ. Mutagen., 8(Suppl. 7): 1-119.

Nagao, M. and Y. Takahashi (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):302-313.

Nakamura, S. et al. (1987). Mutat. Res., 192(4):239-246.

NTP (1992). Technical Report No. 22, "Toxicity Studies of N,N-Dimethylformamide administered by Inhalation to F334/N and B6C3F1 Mice," (November) (HSDB/78).

Parry, J. M. and D. C. Sharp (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program): 468-480.

Purchase, I. F. H. et al. (1976). Nature, 264:624-627.

Purchase, I. F. H. et al. (1978). Br. J. Cancer, 37:873-959.

Rowland, I. and B. Sevem (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):323-332.

Sharp, D. C. and J. M. Parry (1981a). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):491-501.

Venitt, S. and C. Crofton-Sleigh (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):351-360.

Data from these additional sources do not support the "negative" study results summarized above.

Hubbard, S. A. et al. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):361-370.

Trueman, R. W. (1981). Prog. Mutat. Res., 1(Eval. Short-term Tests Carcinog.: Rep. Int. Collab. Program):343-350.

Dobias, L. et al. (1981). Chem. Prum., 31(10):548-551 (CA96:63976).

McGregor, D. B. et al. (1988). Environ. Mol. Mutagen., 11(1):91-118.

Type:	***In vitro* Unscheduled DNA Synthesis Test: No Data were available for MMF**
Supporting Data Type:	***In vitro* Unscheduled DNA Synthesis Test**
Cell Type:	Human embryonic intestinal cells
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S9
Exposure Concentrations:	Highest concentration tested was 9.663 mg/L. Other concentrations were not reported.
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The test compound and positive controls were dissolved in dimethylsulphoxide. Positive control substances used in the test included 4-nitroquinoline-N-oxide and 2-aminoanthracene. S9 mix contained NADP-di-Na-salt, glucose-6-phosphate-di-Na-salt, $MgCl_2$, and KCl. A preliminary toxicity test was conducted to establish the range of concentrations to be used in the DNA repair assay. No toxicity was observed even at the highest concentration of 9.663 mg/mL which was selected as the highest in a series of concentrations of DMF.

Initial Assay: The cells were harvested, sedimented, and suspended in fresh culture medium at a density of $5x10^4$ cells/mL, and samples of this suspension were pipetted into tissue culture Petri dishes containing 3 sterile coverslips. These were incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 hours. The medium was then replaced with arginine-deficient DMEM supplemented with 5% heat inactivated fetal bovine serum and the plates incubated for 24 hours. The medium was then replaced with an arginine-deficient DMEM and the incubation continued for 48 hours more. At the end of this time, the cultures were divided into 2 groups and S9 mix was added to one of the groups. Solutions of hydroxyurea in sterile distilled water and 6-[^{3}H]-thymidine were added to each culture. DMF was dissolved in dimethylsulphoxide and dilutions were made from this stock solution to give the required concentrations. Triplicate wells, with and without S9, received samples of test compound solution. Dimethylsulphoxide was added to the negative control cultures.

After incubation for 3 hours at 37°C in an atmosphere of 5% CO_2 in air, the cultures were repeatedly rinsed in phosphate buffered saline (PBS), which removed loose cells and soluble [^{3}H]-thymidine. They were then incubated for 10 minutes in sodium citrate and finally fixed in methanol:acetic acid for 18 hours. Coverslips were air dried and attached, cells uppermost, to clean microscope slides. Cells were then processed for autoradiography and stained. The stained autoradiographs were examined via microscope. Fifty nuclei were examined for each culture. The data were recorded as the average net grain counts for 3 coverslips ± the standard deviation.

Method 2: Flow 11,000 cells were harvested, sedimented, and suspended in fresh culture medium at a density of $5x10^4$ cells/mL. Samples were dispensed into tissue culture Petri dishes which were incubated in a humid atmosphere of 5% CO_2 in air at 37ºC for 72 hours. The medium was then replaced with arginine-deficient medium supplemented with 5% heat-inactivated FBS and the dishes incubated for 24 hours. The medium was then replaced with an arginine-deficient DMEM and the incubation was continued for another 48 hours. The dishes were then randomly divided into 2 groups and S9 mix was added to one of the groups. Solutions of hydroxyurea and [^{3}H]-deoxyguanosine were added to each dish. DMF was added to give a final

concentration of 9.663 mg/mL.

After a 4-hour incubation at 37ºC in an atmosphere of 5% CO_2 in air, the cultures were washed 3 times with PBS, harvested using a trypsin/EDTA/solution and suspended in saline-EDTA. Cells were disrupted by 30 strokes of a glass pestle in a glass uniform homogeniser, NaCL and sodium lauryl sulphate added, and the mixture incubated for 10 minutes at room temperature. The lysate was then vigorously shaken with phenol-hydroxtquinoline and centrifuged for 15 minutes. The upper, aqueous phase was carefully removed and a sample was mixed with caesium chloride. The solution was poured into centrifuge tubes and overlaid with liquid paraffin. Tubes were centrifuged for 72 hours. Gradients were fractioned by upward displacement with saturated caesium chloride using an ISCO density fractionator, 8 drop fractions were collected on filter discs. The filter discs were immersed for 10 minutes in 2 changes of ice-cold trichloroacetic acid containing sodium pyrophosphate, washed twice in ice-cold hydrochloric acid, and finally once in ethanol. After air drying, the discs were placed in scintillation fluid and analyzed for radioactivity in a liquid scintillation counter. Gradient cells of the DNA from the cells treated with test compound were compared with the profiles of the DNA from cells incubated with 4-nitroquinoline-N-oxide, 2-aminoanthracene, and dimethylsulphoxide.

Several experiments were carried out in the presence of S9 mix in which it was found that no [^{3}H]-deoxyguanosine was incorporated by any of the cultures treated. In the belief that some component of S9 was perhaps metabolizing the [^{3}H]-deoxyguanosine to a derivative which was not incorporated into nucleic acids, the experimental protocol was altered. Cells which had been growing for 72 hours in arginine-deficient medium were treated with hyroxyurea, S9 mix, and test compound, 2-aminoanthracene, or DMSO. After a 3-hour exposure at 37ºC, the incubation medium was removed and the cells were washed twice with PBS. The cells were then covered with new arginine-deficient medium. [^{3}H]-deoxyguanosine was then added and the monolayers were incubated for a further 2.5 hours in the presence of the labeled precursor. The extraction procedure was the same as for those cells without S9.

GLP: No Data

Test Substance: Dimethylformamide, purity 99%

Results:	Negative
Remarks:	In the initial assay involving tritiated thymidine incorporation into non-S phase cells, there was no indication of any increase in the number of silver grains per nucleus at any concentration of DMF. Significant responses were observed in the cells treated with the positive controls. The tritiated deoxyguanosine incorporation assay was used to confirm the results of the first assay. During the course of these experiments, the permeability of both cell lines to deoxyguanosine decreased dramatically. This reduction was aggravated by the addition of S9 mix to the incubation medium. Consequently, the measured incorporation of radioactivity was insufficient to provide a reasonable analysis of the data.
Reference:	National Institute of Occupational Safety and Health (1981). Report No. 33, Inveresk Research International Limited, Contract No. 210-78-0026, "Tier II Mutagenic Screening of 13 NIOSH Priority Compounds" (May 1) (cited in TSCA Fiche OTS0516796).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Unscheduled DNA Synthesis:

Data from these additional sources support the "negative" study results summarized above.

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Type: ***In vitro* Clastogenicity: No Data were available for MMF**

Supporting Data: DMF

Type:	***In vitro* CHO Cytogenetics Assay**
Cell Type:	Chinese Hamster Ovary (CHO) cells
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S9
Exposure Concentrations:	
Method:	No specific test guideline was reported; however, the procedures used in the test followed those reported by Galloway, S. et al. (1985). Environ. Mutagen., 7:1-52 and Galloway, S. et al. (1987). Environ. Mol. Mutagen., 10(Suppl. 10):1-176. CHO cells were incubated with DMF or solvent (dimethylsulfoxide) for induction of sister chromatid exchanges and chromosomal aberrations, both in the presence or absence of S9. Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency that was 20% above the concurrent solvent control value was chosen as a statistically positive result. A single increased dose was considered weak evidence for a positive response. A positive response occurred if 2 or more doses were significantly increased. Chromosomal aberration data are presented as the percentage of cells with aberrations. The dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant difference for 1 dose point and a significant trend were considered weak

	evidence for a positive response. Significant differences for 2 or more doses indicated the trial was positive.
GLP:	Yes
Test Substance:	Dimethylformamide, purity > 99%
Results:	Negative
Remarks:	No additional data.
Reference:	NTP (1992). Technical Report No. 22, "Toxicity Studies on N,N-dimethylformamide in F344/N Rats and B6C3F1 Mice (13-Week Inhalation Studies)" PB93191936 (November).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Clastogenicity:

Data from these additional sources support the "negative" study results summarized above.

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Data from these additional sources do not support the "negative" study results summarized above.

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Koudela, K. and K. Spazier (1979). Cesk. Hyg., 24:432.

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Other References for *In Vitro* Genetic Toxicity:

Data from these additional sources support the "negative" study results summarized above. References include cell transforming studies, DNA repair, and aneuploidy studies.

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Data from these additional sources do not support the "negative" study results summarized above. References include cell transforming studies, DNA repair, and aneuploidy studies.

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Type: ***In vivo* Mouse Micronucleus Assay: No Data were available for MMF**

Supporting Data: DMF

Type:	***In vivo* Mouse Micronucleus Assay**
Species/Strain:	Mice/BALB/c
Sex/Number:	Male/5 per group
Route of Administration:	Intraperitoneal injection
Concentrations:	0, 0.2, 20, 2000 mg/kg
Method:	No specific test guideline was reported; however, procedures generally followed those outlined in Schmid. W. (1976). "The micronucleus test for cytogenetic analysis" in Hollaender. A. (Ed). Chemical Mutagens, Vol. 4, pp. 31-53, Plenum, New York. Mice were 12-13 weeks old at study start. Five animals were used in each treatment group. Positive controls received 100 mg/kg cyclophosphamide. Preparations were made 30 hours after treatment.
GLP:	Unknown
Test Substance:	Dimethylformamide, purity not reported
Results:	Negative

Remarks: The number of cells with micronuclei in each test group can be found in the table below.

Treatment	Cells with micronuclei (%±S.D.)
Negative control	1.6±0.24
Cyclophosphamide	44.2±1.6
DMF 2000 mg/kg	1.8±0.37
DMF 20 mg/kg	1.6±0.40
DMF 0.2 mg/kg	1.8±0.37

Reference: Antoine, J. L. et al. (1983). Toxicology, 26:207-212.
Reliability: Medium because a scientifically defensible or guideline method was used; however, limited study information was available.

Additional References for *In vivo* Mouse Micronucleus Assay:

Data from these additional sources support the "negative" study results summarized above.

Antoine, J. L. et al. (1983). Toxicology, 26(3-4):207-212.

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Data from these additional sources do not support the "negative" study results summarized above.

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Type: ***In vivo* Dominant Lethal Assay: No Data were available for MMF**

Supporting Data: DMF

Type:	***In vivo* Dominant Lethal Assay**
Species/Strain:	Rats/Sprague Dawley
Sex/Number:	Male/10 per group
Route of Administration:	Inhalation
Exposure Duration:	6 hours/day for 5 days
Concentrations:	0, 30, 300 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Each male was caged with 2 females per week for 2 consecutive weeks during the pre-treatment mating period. The females were sacrificed 18 days after the first day of caging with the males and implants observed to indicate pregnancy. Males were considered fertile if at least 1 female was pregnant.

For each DMF chamber, a stream of dry air at room temperature was passed through a bubbler containing DMF. The air flow rate was monitored with a rotameter and was varied to volatilize the appropriate amount of DMF. The vapor-air mixture was diluted to the appropriate concentration with room air prior to entering the exposure chamber. The chamber flow rates were also varied to maintain the proper concentration. Exposure chambers, operated dynamically and were stainless steel and glass of effective volume of 760 L. Atmospheric sampling was conducted and analyzed via an infrared analyzer.

On the last day of the treatment period, at least 2 hours after the last exposure, 2 untreated virgin females were placed in to each male's cage. The male and females remained together for 7 days at which time the females were removed and replaced with 2 new, untreated virgin females. This mating procedure continued for 6 consecutive weeks (post-treatment period). Females removed from the male's cages were sacrificed 18 days after the first day of caging with the male, and implantation data were recorded. Females were considered pregnant when at least 1 uterine implantation site was observed during the gross examination. The number of uterine implantation sites was recorded as well as early resorption sites, late resorption sites, and viable

fetal swellings.

Males were sacrificed at the completion of the study following sacrifice of the last females and a necropsy was performed. For 5 males/group, the seminal vesicles, epididymides, prostate, and testes were preserved and examined histopathologically. Any abnormal lesions or tissue masses observed on any male were also preserved and examined.

TEM (triethylenemelamine) was used as the positive control substance in the study. The males in the positive control group received a single i.p. dose of 0.3 mg/kg 2 hours prior to mating.

Comparisons were made for each post-treatment mating week between the negative control, positive control, and DMF-treated groups. Incidence data were evaluated by the Chi-square test. Absolute data were compared using the F-test and Student's t-test. When variances differed significantly, Student's t-test was appropriately modified using Cochran's approximation.

GLP: No

Test Substance: Dimethylformamide, purity not reported

Results: Negative

Remarks: There was no mortality observed during the study. Pharmacologic and toxicologic observations recorded during the study showed no test-substance related findings.

At the 300 ppm level, DMF-treated males had a slightly lower mean body weight gain during the post-treatment period.

Pregnancy rates and implantation efficiency values for females exposed to DMF-treated males were comparable to the negative control throughout the post-treatment period.

Fetal death data (mean per pregnant female and percentage of total implantation sites) for the DMF-treated groups were slightly higher than control at week 2 (both groups), week 5 (30 ppm group), and week 6 (300 ppm group). At each interval, the increase in fetal deaths in the DMF-treated groups was, in part, attributed to a single female that had uterine implants comprised entirely of early fetal deaths. These increases in fetal deaths were not considered indicative of a dominant lethal mutagenic response since the

second female of the pair mated with the DMF-treated male had high numbers of uterine implants which in most cases were all viable fetal swellings.

There were no treatment-related histopatholgic alterations noted during the study.

Reference: Biodynamics Inc. (1978). Project No. 77-1963, "A Dominant Lethal Inhalation Study of DMF in Rats" (October 31) (cited in TSCA Fiche OTS0516779).

Lewis, S. C. et al. (1979). Environ. Mutagen., 1(2):166.

Cragin, D. W. et al. (1990). Environ. Mol. Mutagen., 15(17):14 (Abstract 44).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vivo* Dominant Lethal Assay:

Data from these additional sources support the "negative" study results summarized above.

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Monsanto (1990). Unpublished Data, "Mutagenic Study with DMF in Albino Mice" (July 28) (TSCA Fiche OTS0526383).

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(CA91:169399b).

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Data from these additional sources support the "negative" study results summarized above.

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Fahmy, M. J. and O. G. Fahmy (1983). Cancer Res., 43:801-807.

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dimethylformamide" (October 23)).