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# Provisional Peer Reviewed Toxicity Values for

# Dimethylformamide
(CASRN 68-12-2)

Superfund Health Risk Technical Support Center
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## Acronyms and Abbreviations

| | |
|---|---|
| bw | body weight |
| cc | cubic centimeters |
| CD | Caesarean Delivered |
| CERCLA | Comprehensive Environmental Response, Compensation and Liability Act of 1980 |
| CNS | central nervous system |
| cu.m | cubic meter |
| DWEL | Drinking Water Equivalent Level |
| FEL | frank-effect level |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| g | grams |
| GI | gastrointestinal |
| HEC | human equivalent concentration |
| Hgb | hemoglobin |
| i.m. | intramuscular |
| i.p. | intraperitoneal |
| IRIS | Integrated Risk Information System |
| IUR | inhalation unit risk |
| i.v. | intravenous |
| kg | kilogram |
| L | liter |
| LEL | lowest-effect level |
| LOAEL | lowest-observed-adverse-effect level |
| LOAEL(ADJ) | LOAEL adjusted to continuous exposure duration |
| LOAEL(HEC) | LOAEL adjusted for dosimetric differences across species to a human |
| m | meter |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| mg | milligram |
| mg/kg | milligrams per kilogram |
| mg/L | milligrams per liter |
| MRL | minimal risk level |
| MTD | maximum tolerated dose |
| MTL | median threshold limit |
| NAAQS | National Ambient Air Quality Standards |
| NOAEL | no-observed-adverse-effect level |
| NOAEL(ADJ) | NOAEL adjusted to continuous exposure duration |
| NOAEL(HEC) | NOAEL adjusted for dosimetric differences across species to a human |
| NOEL | no-observed-effect level |
| OSF | oral slope factor |
| p-IUR | provisional inhalation unit risk |
| p-OSF | provisional oral slope factor |
| p-RfC | provisional inhalation reference concentration |

| | |
|---|---|
| p-RfD | provisional oral reference dose |
| PBPK | physiologically based pharmacokinetic |
| ppb | parts per billion |
| ppm | parts per million |
| PPRTV | Provisional Peer Reviewed Toxicity Value |
| RBC | red blood cell(s) |
| RCRA | Resource Conservation and Recovery Act |
| RDDR | Regional deposited dose ratio (for the indicated lung region) |
| REL | relative exposure level |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| RGDR | Regional gas dose ratio (for the indicated lung region) |
| s.c. | subcutaneous |
| SCE | sister chromatid exchange |
| SDWA | Safe Drinking Water Act |
| sq.cm. | square centimeters |
| TSCA | Toxic Substances Control Act |
| UF | uncertainty factor |
| µg | microgram |
| µmol | micromoles |
| VOC | volatile organic compound |

# PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR DIMETHYLFORMAMIDE (CASRN 68-12-2)

## Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

## Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in

this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

**Questions Regarding PPRTVs**

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

## INTRODUCTION

No RfD for dimethylformamide is listed on IRIS (U.S. EPA, 2007) or the Drinking Water and Health Advisories list (U.S. EPA, 2004). The HEAST (U.S. EPA, 1997) lists a subchronic oral RfD of 1 mg/kg-day and a chronic oral RfD of 0.1 mg/kg-day based on the derivation of an Allowable Daily Intake (ADI) for 'rats' [sic] in a Health and Environmental Effects Profile (HEEP) on dimethylformamide (U.S. EPA, 1986). The HEEP based the provisional acceptable daily intake (ADI) on a no-observed-adverse-effect level (NOAEL) of 96 mg/kg-day for hepatic effects in [male] CD-1 mice exposed to dimethylformamide in the diet for 119 days (Becci et al., 1983). In the chronic ADI derivation, an uncertainty factor of 1000 (10 to extrapolate from animals to humans, 10 to protect sensitive individuals and 10 for the use of a subchronic study) was applied to the NOAEL. The HEAST dropped the last uncertainty factor of 10 to derive the subchronic RfD. No additional relevant documents for dimethylformamide are included in the chemical assessments and related activities (CARA) list (U.S. EPA, 1991a, 1994a).

IRIS (U.S. EPA, 2007) lists a chronic reference concentration (RfC) of 0.03 mg/m$^3$ for dimethylformamide. The HEAST (U.S. EPA, 1997) lists a subchronic RfC of 0.03 mg/m$^3$, adopted from the chronic RfC of the same value on IRIS (U.S. EPA, 2007). The chronic RfC was based on co-principal studies that evaluated hepatic effects in humans following occupational exposure to dimethylformamide: Cirla et al. (1984), which evaluated 100 exposed/referent pairs and Catenacci et al. (1984), which evaluated 54 exposed/referent pairs. Catenacci et al. (1984) found no hepatic enzyme increases in workers exposed to 18 mg/m$^3$ (8-hour TWA), but the power

of the study was considered insufficient to use this NOAEL as the basis for the risk assessment (U.S. EPA, 2007). Instead, the RfC was based on a lowest-observed-adverse-effect level (LOAEL) of 22 mg/m$^3$ (mean concentration) in the study of Cirla et al. (1984) for digestive disturbances (disulfiram-like alcohol intolerance and dyspepsia) and minimal (i.e., not statistically significant) hepatic changes suggestive of liver abnormalities (liver enlargement, elevated serum aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) in workers exposed for an average of 5 years (range 1-15 years). The LOAEL was adjusted for duration by multiplying by the ratio of the reference breathing volume for light activity during 8-hour occupational exposure to the reference daily breathing volume (10/20 m$^3$/day) and by the ratio of workdays to weekdays (5/7). An uncertainty factor of 300 (10 to protect sensitive individuals, 10 to account for use of a LOAEL and 3 combined for the lack of reproductive toxicity data and the less than chronic duration of exposure) was applied to the duration-adjusted LOAEL of 7.9 mg/m$^3$ to arrive at the RfC of 0.03 mg/m$^3$. CalEPA (2000) derived a chronic reference exposure level (REL) of 0.08 mg/m$^3$ for dimethylformamide based on the same adjusted LOAEL of 7.9 mg/m$^3$, but applying a cumulative uncertainty factor of 100 (3 for the use of a LOAEL, 3 for the subchronic duration and 10 for intraspecies variation). ACGIH (2005a, 2005b), NIOSH (2005), and OSHA (2006) have all established occupational exposure limits of 30 mg/m$^3$ (10 ppm, 8-hour time-weighted average [TWA]) for dimethylformamide to protect against toxicity to the liver, central nervous system, kidney and heart, and irritation of the eyes and respiratory tract.

No cancer assessment for dimethylformamide is available in IRIS (U.S. EPA, 2007), the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004), or the HEAST (U.S. EPA, 1997). The HEEP (U.S. EPA, 1986) assigned dimethylformamide to Group D, not classifiable as to human carcinogenicity, based on inadequate evidence in humans and laboratory animals and overwhelming evidence that the compound is not mutagenic or genotoxic. The most recent assessment by IARC (1999, 2002) concluded that dimethylformamide is not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence in humans, evidence suggesting a lack of carcinogenicity in experimental animals and consistently negative results for genotoxicity in well-controlled *in vitro* and *in vivo* assays. ACGIH (2005a, 2005b) added an A4 notation to the threshold limit value [TLV]-TWA for dimethylformamide to indicate that the compound is not classifiable as a human carcinogen.

ATSDR (2007) has not reviewed the toxicology of dimethylformamide. An Environmental Health Criteria document (WHO, 1991), a Concise International Chemical Assessment Document (CICAD) (WHO, 2001), a Priority Substances List Assessment Report (Health Canada, 2001) and IARC (1989, 1999) monographs on dimethylformamide, a toxicity risk assessment of dimethylformamide (Long and Meek, 2001), a toxicity review on miscellaneous organic nitrogen compounds (Trochimowicz et al., 2001) and the NTP (2006a, 2006b) management status report and health and safety report for dimethylformamide were consulted for relevant information. Literature searches were conducted for the period from 1985 to October 2002 to identify data relevant for the derivation of a provisional RfD, RfC and cancer assessment for dimethylformamide. The following databases were searched: TOXLINE, MEDLINE, CANCERLIT, NTIS/BIOSIS, RTECS, HSDB, GENETOX, CCRIS, TSCATS, EMIC/EMICBACK and DART/ETICBACK. An update search of the following databases was conducted in November 2005: TOXLINE Special (NTIS subfile), TOXCENTER (BIOSIS

subfile), MEDLINE (plus PubMed cancer subset), TSCATS, CCRIS, DART/EMIC, GENETOX, HSDB, RETCS and Current Contents (June to November, 2005). An update search of the PubMed database was conducted in September 2007.

## REVIEW OF PERTINENT DATA

### Human Studies

Several epidemiological studies have investigated effects on the liver in workers exposed to dimethylformamide.

Cirla et al. (1984) studied 100 workers exposed to a mean concentration of 22 mg/m$^3$ of dimethylformamide (range of 8-58 mg/m$^3$, determined with personal air sampler) for an average of 5 years (range of 1-15 years), and compared the results with those obtained from 100 pair-matched referent controls. The authors accounted for alcohol ingestion, cigarette smoking and caffeine in selecting the matching characteristics. The population studied was male, with a mean age of 36 years (range of 21-56 years of age). Their work history was carefully verified, and the possibility of peak exposures to dimethylformamide was ruled out. The workers were evaluated by means of a questionnaire of subjective complaints, medical examination and laboratory studies, including aminotransferase and gamma-glutamyl transpeptidase (GGT) levels. Dimethylformamide-exposed workers complained more often of headache, dyspepsia, nonspecific cardiac distress and digestive impairment indicative of hepatic functional impairment. Symptoms of respiratory irritation that were significantly increased in the dimethylformamide exposed group included watery eyes, cough and dry throat. Several of the exposed workers complained of a disulfiram-type reaction upon alcohol ingestion. Among exposed workers, the prevalence of abnormally high GGT levels (25/100) was statistically significantly increased versus controls (10/100). Prevalence was also higher in the exposed group for other indicators of hepatotoxicity, including liver enlargement (20/100 vs 16/100), elevated AST (9/100 vs 3/100), and elevated ALT (12/100 vs 8/100), although none of these differences achieved statistical significance. Within the dimethylformamide exposed group, there were no differences between a subgroup with no dermal exposure and a subgroup with potential dermal exposure (data not presented), indicating that exposure to dimethylformamide via the dermal route was not a significant confounder. The average exposure level of 22 mg/m$^3$ in this study is a LOAEL for digestive disturbances and mild liver abnormalities resulting from occupational exposure to dimethylformamide.

Catenacci et al. (1984) examined hepatic function in workers exposed to low levels of dimethylformamide. They studied AST, ALT, GGT and alkaline phosphatase (AP) levels in 54 workers who had been employed in an acrylic fiber plant for more than 5 years. The workers were divided into two groups; the first group of 28 subjects was exposed to an 8-hour TWA concentration of 18 mg/m$^3$ (range of 12-25 mg/m$^3$) of dimethylformamide, and the second group of 26 subjects was exposed to an 8-hour TWA concentration of 3 mg/m$^3$ (range of 1-5 mg/m$^3$) of dimethylformamide. The control group consisted of 54 workers who were never exposed to solvents. No significant difference was observed between either of the exposed groups and the

controls for any of the parameters tested. Thus, the 8-hour TWA concentration of 18 mg/m$^3$ was a NOAEL for hepatotoxicity in this study. However, the power of this study to detect a difference in enzyme levels was limited because only 54 matched pairs were used.

Fiorito et al. (1997) evaluated liver function in 75 workers exposed to dimethylformamide at an 8-hour TWA of 20 mg/m$^3$ (range 2-40 mg/m$^3$) in a synthetic leather factory for up to 3.8 years. From the original pool of 86 workers, 11 were excluded from the study based on nonoccupational liver complaints, recent drug therapy or high alcohol consumption. The control group included 75 unexposed workers similar in age, sex, social status and residence. All subjects underwent a complete physical examination with liver function tests for serum enzymes, bile acids, bilirubin, serum cholesterol and triglycerides and hepatitis markers. Solvent absorption was estimated from N-methylformamide (NMF) concentrations in pre- and post-shift urine samples from a subset of 22 subjects in the same work shift. Fifty percent of exposed workers reported gastrointestinal symptoms (stomach pain, nausea, loss of appetite). Alcohol consumption was significantly lower in the exposed group, because alcohol use was reported to cause symptoms in the workplace. Forty percent of exposed workers reported symptoms of alcohol intolerance: face flushing (38%), palpitation (30%), headache (22%), dizziness (22%), body flushing (15%) and tremors (14%). Mean values of liver enzymes (AST, ALT, GGTP, AP) were statistically significantly increased in exposed workers; there were no effects on serum cholesterol or other tested indices. In addition, the prevalence of workers with abnormally high values was significantly increased for ALT (>10%), AST (>19%) and GGT (>12%) in comparison to controls (all <4%). Multivariate analysis showed that ALT, AST and GGT levels were not significantly correlated with age or alcohol consumption, and that cumulative exposure to dimethylformamide (approximated as work seniority) was a stronger predictor of serum enzyme levels than serum cholesterol levels or body mass index, which were also correlated with enzyme levels. In this study, the average exposure level of 20 mg/m$^3$ was a LOAEL for hepatic effects in exposed workers.

Cai et al. (1992) evaluated health effects of dimethylformamide in workers in a polyurethane plant. A total of 207 workers (114 men and 93 women) in five different departments were exposed to mean 8-hour TWA concentrations between 0.2 and 9.1 ppm (0.6 to 28 mg/m$^3$) with an overall group average of 4.5 ppm (13.7 mg/m$^3$) and compared to 143 non-exposed workers (67 men and 76 women) in the same plant. The duration of exposure was "several years." Subjects were questioned as to the incidence of subjective symptoms during the preceding 3-months and were evaluated for hematology and serum biochemistry (including AP, ALT, AST, GGT and bilirubin). Hematology and serum biochemistry values in exposed workers were not different from those of the controls. However, the prevalences of subjective symptoms relating to the digestive system (nausea, vomiting, dry mouth and abdominal pain) and other symptoms (eye irritation, dimmed vision, nasal irritation, sore throat, dizziness, tightness in chest, breath shortage and unusual taste sensation) were significantly higher in the exposed group than in the controls. Analysis by department (exposure level) found that the prevalence of self-reported reduced alcohol tolerance was significantly elevated in subjects exposed to levels of 2 ppm (6 mg/m$^3$) or above, but not average exposures of 0.1-0.6 ppm (0.3-1.8 mg/m$^3$). This study identified a LOAEL of 6 mg/m$^3$ and a NOAEL of 1.8 mg/m$^3$ based on subjective symptoms of hepatic effects in workers exposed to dimethylformamide.

Wrbitzky (1999) evaluated liver function in 126 male employees exposed to dimethylformamide and in 53 unexposed males in a factory producing synthetic fibers. Workers were exposed to dimethylformamide at a median concentration of 1.2 ppm (3.6 mg/$m^3$); the range was <0.1 ppm (the detection limit) to 37.9 ppm (<0.3-113.7 mg/$m^3$). Subjects were evaluated by a medical questionnaire, liver function parameters (ALT, AST, GGT) and hepatitis antibody screening. The prevalence of subjective symptoms of alcohol intolerance (flush symptoms, voluntary reduction in alcohol consumption) was significantly higher in the exposed group. The exposed group had significantly elevated levels of AST and GGT. However, when the effects of alcohol consumption and levels of exposure to dimethylformamide on the liver index [(ranking indices for GGT + AST + ALT) divided by 3 (number of parameters)] were evaluated statistically, the effect of alcohol ($p<0.001$) was found to be more pronounced than the influence of dimethylformamide ($p=0.043$). For the subset of workers who did not consume alcohol, there was no difference in the liver index between nonexposed workers and exposed workers, irrespective of exposure level. In this study, alcohol consumption is a confounding factor. Therefore, a reliable NOAEL or LOAEL for dimethylformamide is not provided by this study.

Lauwerys et al. (1980) studied 22 workers who were exposed to 1-46.3 mg/$m^3$ dimethylformamide for an average of 5.3 years, and compared the results with those obtained from 28 control workers. They found no significant difference in liver function tests (ALT, AST, OCT, GGT, AP, bilirubin) when the exposed workers were compared to the control workers. However, signs of alcohol intolerance were observed in the workers at dimethylformamide levels below 30 mg/$m^3$. Similarly, Yonemoto and Suzuki (1980) found no effects on AST, ALT, AP or GGT in 11 workers exposed to 3-15 mg/$m^3$of dimethylformamide, but the workers reported episodes of alcohol intolerance.

Redlich and coworkers investigated an outbreak of liver disease in workers employed in a fabric coating factory where dimethylformamide, along with other solvents, was mixed with polyurethane and used to coat fabrics (Redlich et al., 1987a,b; 1988; Riely et al., 1988). Exposure levels were not quantified, but workers were exposed to large quantities (approximately 15-20 55-gallon drums per week) of dimethylformamide in poorly ventilated areas without appropriate skin protection. Average duration of employment was 40 months, but 15 workers were employed for 3 months or less. Fifty-eight of the 66 workers participated in the study. Elevations of either AST or ALT levels were observed in 36 of the 58 employees, with 19 of these workers showing increases of twice the normal values, which the researchers considered to be indicative of liver disease. The liver enzyme levels correlated with job classification; 11 of 12 nonproduction workers had normal liver enzymes while 35 of 46 production workers had some elevation in liver enzyme levels. These changes in liver enzyme levels were verified by pathological evidence of liver disease revealed in liver biopsies taken from seven workers. The changes seen were variable, but consisted mainly of microvesicular and macrovesicular steatosis, spotty necrosis and evidence of diffuse regeneration. None of the biopsies showed changes indicative of alcoholism (i.e., fibrosis or cirrhosis). Furthermore, questionnaire results from 46 workers revealed the following symptoms: gastrointestinal discomfort (anorexia, abdominal pain or nausea) in 31 respondents, central nervous system solvent intoxication (headaches, dizziness) in 18 respondents and alcohol intolerance characterized by a disulfiram type reaction (facial flushing and palpitations after alcohol intake) in 11 respondents. The results demonstrate that occupational

exposure to high levels of dimethylformamide can result in liver disease. The data are not sufficient to determine whether the effects were reversible or whether chronic liver disease can result from long-term exposure. Also, concurrent exposure to other solvents, including methylethylketone, toluene, 1,1,1-trichloroethane and dichlorobenzene may have potentiated the hepatotoxicity of dimethylformamide.

A number of additional occupational and case studies report the occurrence of hepatic effects in exposed workers. These studies are limited by small sample size and uncharacterized exposure levels. For many, translations were not available, so summaries from secondary sources or abstracts are discussed below. Massmann (1956a) reported that all liver function tests were normal in 24 workers exposed to dimethylformamide at levels that were generally below 10 ppm for 3-24 months. Reinl and Urban (1965) studied 12 workers exposed to dimethylformamide levels that were generally <20 ppm for 1-32 weeks and reported the occurrence of alcohol intolerance, vomiting, hepatomegaly, jaundice, urobilinuria and elevated serum aminotransferases in several of these workers. No increase in serum aminotransferases was observed in workers exposed to an unknown concentration of dimethylformamide for 6 months (Di Lorenzo and Graziolo, 1972). Tolot et al. (1968) reported that a liver biopsy performed 3 months after an acute accidental exposure to an unspecified level of dimethylformamide showed vacuolization of the parenchymal cells. Abdominal pain, nausea and vomiting, and ethanol intolerance were observed in four workers exposed to an unknown concentration of dimethylformamide (Chary, 1974). Qian et al. (2007) reported changes in ALT and GGT in workers exposed to dimethylformamide (concentrations not reported) in a synthetic leather factory. Luo et al. (2005) examined liver function parameters and incidence of chronic liver disease (as determined by ultrasonography) in workers (industry not reported) in a "high" dimethylformamide exposure group (average workplace concentration – 23.9 ppm; range – 5.2-86.6 ppm) to workers in a "low" exposure group (average – 2.41 ppm; range – 0.9-4.3 ppm); the incidence of abnormal liver function tests was 13/44 (29.6%) in the high-exposure group and 2/22 (9.1%) in the low-exposure group; chronic liver disease was reported in 7/44 (15.9%) of the high-exposure workers and 0/22 of the low-exposure workers.

Taken together, these studies demonstrate that dimethylformamide exposure is associated with hepatic toxicity in humans. Subjective evidence of liver toxicity (e.g., digestive impairment, alcohol intolerance) is often seen at exposure concentrations below those that cause elevations in serum enzymes of hepatic function, and thus may serve as a more sensitive indicator of dimethylformamide-induced hepatic toxicity. The reports indicate that dimethylformamide concentrations as low as 6-20 $mg/m^3$ may produce these effects. A reliable NOAEL has not been identified.

One report was located on the reproductive effects of dimethylformamide in humans (Farquharson et al., 1983). The researchers described the occurrence of three unexplained cases of small-for-date third trimester intrauterine deaths in female quality control analysts working in the pharmaceutical industry. This represents a 30% stillbirth rate as compared with the average rate of 0.26% for the general population of that area. The authors concluded that this occurrence was not likely due to chance, but cannot be attributed solely to dimethylformamide because these women were exposed to other agents in addition to dimethylformamide.

IARC (1999) reviewed carcinogenicity studies in humans exposed to dimethylformamide. Case reports of testicular cancer among 7 out of 680 F4 Phantom jet aircraft repair technicians exposed to a solvent containing 80% dimethylformamide (Ducatman et al., 1986) and 3 out of 51 workers in a leather tannery (Levin et al., 1987; Calvert et al., 1990) led to several epidemiological studies evaluating the carcinogenicity of dimethylformamide. Chen et al. (1988) studied cancer incidence among 2530 active employees of the DuPont company with potential exposure to dimethylformamide during 1950-1970 in Virginia and 1329 employees with exposure to dimethylformamide and acrylonitrile in a plant in South Carolina. For all workers exposed (alone or with acrylonitrile), the standardized incidence ratio for all cancers combined was 1.1 (95% confidence interval [CI]: 0.9-1.4). Only one case of testicular cancer was identified among 3859 workers (1.7 expected). Walrath et al. (1989) conducted a case-control study for several cancer types among workers from four DuPont plants. Geometric means for air measurements of dimethylformamide ranged from a low of <1 ppm (3 $mg/m^3$) to a moderate level around 10 ppm (30 $mg/m^3$). Mantel-Haenszel odds ratios for exposed individuals were 1 (n=3) (90% CI: 0.4-2.3) for testicular cancer, indicating no increase. Odds ratios for testicular cancer were 0.9 (90% CI: 0.1-8.6) for the low-exposure group and 3.1 (90% CI: 0.8-11.9) for the moderate-exposure group. IARC (1999) concluded that these studies do not provide convincing evidence for the carcinogenicity of dimethylformamide in humans.

No data were located regarding the toxicity or carcinogenicity of dimethylformamide in humans exposed orally.

**Animal Studies**

*Oral Exposure*

The toxicity of dimethylformamide has been analyzed extensively in animals exposed orally or by inhalation. Oral toxicity data include subchronic dietary studies in rats and mice (Becci et al., 1983; Kennedy and Sherman, 1986), subchronic drinking water studies in rats and gerbils (Savolainen, 1981; Elovaara et al., 1983; Llewellyn et al., 1974), a reproductive drinking water study in mice (Fail et al., 1998; NTP, 1992a) and developmental gavage studies in rabbits, rats and mice (Hellwig et al., 1991; Saillenfait et al., 1997; Fritz and Giese, 1990; Merkle and Zeller, 1980). No chronic duration oral studies were located.

Becci et al. (1983) exposed groups of 25 male and 25 female Wistar rats to dimethylformamide in the diet at concentrations of 0, 215, 750 or 2500 ppm for 104 days. Reported mean compound consumption was 0, 18, 61 or 210 mg/kg-day for male rats and 0, 20, 69 or 235 mg/kg-day for female rats. Endpoints that were examined throughout the study included behavior and clinical signs (daily), body weight and food consumption (weekly), and hematology, clinical chemistry and urine indices (five animals/sex/group after 30 and 90 days). Hematology indices included hematocrit, erythrocyte count, total and differential leukocyte counts, platelet count, reticulocyte count, erythrocyte sedimentation rate and hemoglobin level. Clinical chemistry indices included levels of glucose, urea nitrogen, total protein, bilirubin, sodium, potassium, SGOT (serum AST), SGPT (serum ALT) and alkaline phosphatase; serum cholesterol and phospholipids were not assessed. Urine indices included pH, specific gravity,

color, gross and microscopic appearance, ketones, protein, bilirubin and occult blood. Endpoints examined at the end of the exposure period included ocular condition, organ weights (10 organs) and histopathology (25 organs and tissues in all high-dose and control animals and five animals/sex in low- and mid-dose groups).

Body weight gain was significantly ($p<0.05$) decreased in the high-dose male and female rats over the course of the study (8.8 and 12.1% less than controls, respectively). Mean food consumption per week was significantly decreased in high-dose males (5.3% less than controls) and mid- and high-dose females (2.5 and 3.4%, respectively). There was no significant effect on food conversion (gram weight gain/100 grams food consumed), leading the authors to suggest that the reduced food consumption (and consequently body weight gain) might have been due to lower palatability of the feed rather than toxicity *per se.* Clinical chemistry, urinalysis and hematological parameters were within normal ranges for rats, although a few sporadic changes achieved statistical significance (decreased hematocrit in low-dose males and increased sodium in high-dose males). High-dose (210 mg/kg-day) males had significantly increased relative, but not absolute, weights of brain and testes (11.1 and 16.9% higher than controls, respectively). Absolute liver weight was increased in females at 235 mg/kg-day (22.3% higher than controls) and relative liver weight was increased in females at 69 and 235 mg/kg-day (9.8 and 29.5%, respectively) and males at 210 mg/kg-day (16.0%). No histological changes were observed in the liver, brain, testes or other organs or tissues. The authors concluded that the increases in relative brain and testes weights are most likely physiological responses to the decreased feed intake. In the absence of any hepatic histopathology or abnormal serum transaminase and alkaline phosphatase levels, the observed increases in liver weight are considered to be an adaptive, rather than adverse effect. The decreases in body weight are not considered to be adverse because the reduction was small (8.8-12.1%) and possibly due to palatability-related decreased food consumption. Due to the lack of clear evidence of an adverse effect on the liver and other organs, the high dose of 210-235 mg/kg-day is classified as a NOAEL.

Becci et al. (1983) similarly exposed groups of 30 male and 30 female CD-1 mice to 0, 160, 540 or 1850 ppm dimethylformamide in the diet for 119 days. Reported mean compound consumption was 0, 22, 70 or 246 mg/kg-day for males and 0, 28, 96 or 326 mg/kg-day for females. Endpoints were the same as in the rat part of the study summarized above (although clinical chemistry was only evaluated after 90 days). No treatment-related effects were observed on body weight gain, food consumption, food conversion, clinical signs, behavior, ophthalmic condition or urinalysis indices. Sporadic hematological changes occurred in females (significantly increased hemoglobin at low-dose and decreased hematocrit at high-dose) but were within normal historical ranges for mice and not considered treatment-related. Serum ALT levels were significantly ($p<0.05$) increased in the high-dose females (66.7% higher than controls; low- and mid-dose values not reported), although still within the historical normal range. No differences in clinical chemistry or hematological parameters were observed in male mice. Absolute and relative weights of the thyroid (11.9 and 15.8% higher than controls, respectively) and adrenal glands (20.0 and 23.1%) were significantly ($p<0.05$) increased in high-dose females. Absolute liver weight was significantly ($p<0.05$) increased in high-dose males and females (30.0 and 25.0% higher than controls, respectively), and relative liver weight was significantly increased in mid- and high-dose females (15.7 and 29.4%, respectively) and high-dose males

(24.5%). Histological examinations showed slight hepatocytomegaly in a majority of high-dose males and females (incidences and other details not reported), but no changes in the thyroid, adrenals or other organs or tissues. The toxicological significance of the increased thyroid and adrenal weights is unclear due to the lack of histopathological changes in these organs. Based on the increases in liver weight, hepatocytomegaly and serum ALT in high-dose mice, 246-326 mg/kg-day is a LOAEL for hepatic effects. The NOAEL is 70-96 mg/kg-day, the dose that caused increased liver weight without histological or serum enzyme changes.

Kennedy and Sherman (1986; Haskell Labs, 1960) fed groups of weanling CD rats (6/sex/group) diets containing 1% peanut oil plus 0, 200, 1000 or 5000 ppm of dimethylformamide (purity >99%) for 93-94 days. Based on reported average weekly intakes of dimethylformamide (Haskell Labs, 1960), the average calculated doses are 0, 16, 77 and 426 mg/kg-day for males and 0, 17, 85 and 422 mg/kg-day for females. Rats were examined daily for mortality and clinical signs; body weights were recorded before exposure, three times per week for the first 3 weeks and twice per week thereafter. Food consumption was measured weekly. Hematology (red cell count, total and differential white cell counts, hemoglobin concentration, hematocrit, red cell diameter, number of nucleated red cells per 100 white cells) and serum alkaline phosphatase levels were evaluated at 30, 60 and 90 days. Serum levels of SGOT (AST), *p*-phenylenediamine oxidase, cholesterol and phospholipids, as well as liver fat content, were determined at 90 days. At termination, all rats were necropsied, major organs were weighed (adrenal, brain, kidney, liver, lung, spleen and testis), a limited number of tissues were examined for histopathology (organs that were weighed and ovary, heart, pancreas, stomach and small intestine) and bone (tibia) length was measured.

Overall body weight gain was 12.2 and 11.4% lower than controls in high-dose males and females, respectively, mainly due to average weight gain reductions of 20.9% in the males during weeks 1-5 and 39.1% in the females during weeks 1-2. Food consumption was reduced in the high-dose males during weeks 1-6 (average 4.1% less than controls) and high-dose females during weeks 1-5 (average 13.2%). Food efficiency (gram weight gain/gram food consumed) was reduced in the high-dose males during weeks 1-5 (average 16.0% less than controls) and high dose females during weeks 1-2 (average 28.3%). Hematological alterations, observed at 90 days, included significantly reduced erythrocyte counts in males and females at 1000 ppm (13.2 and 4.9% lower than controls, respectively) and 5000 ppm (7.3 and 21.5% lower), and increased leucocyte counts in males at 1000 and 5000 ppm (10.0 and 42.7% higher than controls, respectively) and females at 5000 ppm (58.2% higher). Serum cholesterol was increased in males at 5000 ppm and females at $\geq$1000 ppm; levels at 200, 1000 and 5000 ppm were 25.2% lower, 2.8% lower and 37.4% higher than controls in males, and 2.9% lower, 51.4% higher and 84.8% higher than controls in females. Serum phospholipids were increased in both sexes at 5000 ppm; levels at 1000 and 5000 ppm (not tested at 200 ppm) were 4.7% lower and 29.9% higher than controls in males, and 5.9 and 24.3% higher than controls in females. Liver fat (% dry weight) was decreased in males at $\geq$1000 ppm and females at 5000 ppm; levels at 200, 1000 and 5000 ppm were 10.9% lower, 31.5% lower and 34.8% lower than controls in males, and 5.0% higher, 10.0% higher and 17.5% lower than controls in females. Absolute and relative liver weights were significantly increased in both sexes at $\geq$1000 ppm; compared to controls at 200, 1000 and 5000 ppm, absolute liver weights were 6.8, 11.6 and 11.7% higher in males and 11.6, 26.3 and 24.0%

higher in females, and relative liver weights were increased 4.0, 12.5 and 24.4% higher in males and 1.8, 13.9 and 31.5% higher in females. Histological changes were limited to the liver and consisted of a slight variation in size and staining quality of the nuclei in the hepatocytes, which were classified as barely perceptible liver injury, in 3/6 males and 5/6 females at 5000 ppm (Haskell Labs, 1960). The hepatocellular changes were further interpreted as increased mitosis and cellular enlargement (Kennedy and Sherman, 1986).

The main target organ was the liver, as shown by the increases in liver weight and serum cholesterol and decrease in liver fat at ≥1000 ppm, and increases in serum phospholipids and histological alterations at 5000 ppm (Kennedy and Sherman, 1986; Haskell Labs, 1960). Assessment of the changes in cholesterol, phospholipids and liver fat is complicated by the small numbers of animals, wide intergroup variations in mean values, unreported standard deviations and insufficient information on normal ranges. Although increases in serum cholesterol are possibly indicative of a disruption in liver function, and were also found in 13-week inhalation studies in rats and mice (Lynch et al., 2003; NTP, 1992b; Senoh et al., 2003), the findings in the oral study are inconclusive because the value in low-dose males was much lower than in the control group, the male and female control values appear to be unusually high for CD rats, the increases were not pronounced, and ranges of normal values could not be located for CD rats of similar age (≈16 weeks old) (CCAC, 2006; Charles River Laboratories, Inc., 1994). The increases in serum phospholipids and decreases in liver fat are similarly inconclusive due to variability in mean values, incompletely reported statistics, small responses, and lack of information on normal ranges. Consequently, based on increases in liver weight and slight histopathology, the LOAEL is 5000 ppm (422-426 mg/kg-day). The NOAEL is 1000 ppm (77-85 mg/kg-day), the highest dose that caused increased liver weight without histological alterations.

Llewellyn et al. (1974) reported that 4/12 female Mongolian gerbils exposed to dimethylformamide in drinking water at 10,000 mg/L died within 200 days. Based on drinking water consumption data, the mean total dose was reported by the study authors as >100,000 mg/kg (>500 mg/kg-day, data not shown). Microscopic examination revealed necrotic lesions in the livers of all the gerbils that died. At a drinking water concentration of 17,000 mg/L, one-half of the gerbils died within 80 days, and the cumulative dose was 90,206 mg/kg (1128 mg/kg-day). At concentrations of 34,000 and 66,000 mg/L, one-half of the gerbils died within 6 and 3 days, respectively. Nearly all of the animals of these higher exposure groups had liver lesions including diffuse zones of necrosis, abnormal numbers of mitotic figures and many Kupffer cells. It should be noted that individual water consumption varied widely within groups.

Additional subacute (Kennedy and Sherman, 1986) to subchronic (Itoh et al. 1987) duration gavage studies also provide evidence of more severe liver toxicity at relatively high bolus doses of dimethylformamide, and relatively mild histopathological changes in a 2-week gavage study using doses that caused hepatocellular hypertrophy in subchronic dietary and drinking water studies. A subacute toxicity study in which male CD rats were administered 0 or 450 mg/kg-day of dimethylformamide (as a 10% solution in water) for nine doses over a period of 2 weeks reported that the treated rats showed “discomfort” after dosing, and their weight gain was depressed relative to controls (Kennedy and Sherman, 1986). Histopathological examination of the livers of the three treated rats sacrificed after the last dose revealed variation in size and

staining of liver nuclei, frequent mitotic figures and some hepatic cells containing two nuclei. No histopathological changes were seen in the three treated rats that were sacrificed after an 11-day recovery period. Gavage treatment of F344 rats (8/sex/group) with 708 or 945 mg/kg of undiluted dimethylformamide once weekly for 12 weeks resulted in hepatic fibrosis and severe hepatic necrosis in both sexes, and decreased body weight gain and depressed activity in males (Itoh et al., 1987). Serum ALT was elevated less than 2-fold in males and unaffected in females, but blood was obtained for clinical chemistry 1 week after the last dose, just prior to necropsy.

Fail et al. (1998; NTP, 1992a) evaluated the effects of N,N-dimethylformamide (purity >99%) on fertility and reproduction in Swiss CD-1 mice using the Reproductive Assessment by Continuous Breeding Protocol. The authors cited previous publications for the study methods (Lamb, 1985; Chapin and Sloane, 1997) and the protocol is also described online (NTP, 2002). In a 2-week range-finding study, groups of eight mice/sex were exposed to drinking water containing dimethylformamide at concentrations of 0, 2500, 5000, 7500, 10,000 or 15,000 ppm. Animals were examined for mortality and clinical signs, but the frequency of observation was not reported. Body weights and food and water consumption data were recorded weekly. Increased mortality was observed in males exposed at $\geq$10,000 ppm and females at 15,000 ppm. Body weight was reduced in surviving mice exposed at 15,000 ppm. Decreased water consumption was observed in both sexes, but the affected groups were not reported; results for food consumption were not reported. On the basis of the range-finding study, drinking water concentrations of 0, 1000, 4000 and 7000 ppm were selected for the continuous breeding study.

In the continuous cohabitation study, Fail et al. (1998; NTP, 1992a) gave untreated drinking water to 40 breeding pairs of Swiss CD-1 mice and drinking water containing 1000, 4000 or 7000 ppm of dimethylformamide to groups of 20 breeding pairs for a 1-week pre-cohabitation period and a 14-week cohabitation period ($F_0$ generation). As estimated from the reported dose ranges, the mean doses were 0, 185, 610 and 1049 mg/kg-day for $F_0$ males and 0, 225, 788 and 1427 for $F_0$ females. Body weights and food and water consumption were monitored for $F_0$ mice on weeks 1, 8 and 16. Other endpoints in the cohabitation study included the litter interval, number of litters/pair, the number, sex and weight of pups per litter, external malformations of pups, and the dam body weight on postnatal day (PND) 0. After week 16 of exposure, the breeding pairs were separated and $F_0$ females were allowed to deliver and rear the final litter until PND 21. On PND 0, 4, 7, 14 and 21 of lactation, randomly selected $F_1$ pups were weaned and saved for the $F_1$ fertility assessment (described below). Following the cohabitation period, crossover mating trials were conducted using $F_0$ high-dose males mated with control females (dosed male group), high-dose females mated with control males (dosed female group) and a control group. Beginning at week 23, breeding pairs were cohabited (untreated) until the appearance of a vaginal copulatory plug or up to 1 week. At week 24, mice were housed separately and dosing was resumed. Upon delivery, each crossover litter was evaluated for lethality, gestation length, sex, number and weight of pups and weight of dams. Selected litters were evaluated for skeletal malformations and for visceral malformations of the head. After all crossover litters had been delivered, vaginal smears were collected from control and 7000 ppm $F_0$ females for 12 days. At week 29, all $F_0$ mice were weighed and necropsied. The following organ weights were measured: liver and paired kidney/adrenal in both sexes, right testis, right epididymis, prostate and seminal vesicles with coagulating glands in males and right ovary with

attached oviducts in females. Histopathologic evaluations were conducted on any gross lesions, livers with gross lesions, right and left kidney/adrenals, right testis and epididymis, prostate, seminal vesicles and ovary. [Fail et al. (1998) state that all livers were examined histopathologically, but NTP (1992a) clearly states that only the livers with gross lesions were examined histopathologically.] Sperm evaluations included assessments of motility, concentration and morphology (right testis/epididymis) and number of homogenization-resistant spermatid heads (left testis/epididymis).

In the cohabitation study, exposure of $F_0$ mice to dimethylformamide had no effect on mortality in either sex (Fail et al., 1998; NTP, 1992a). Treatment had no effect on body weight in males, but caused significant reductions in females; effects in females were observed in the latter half of the study at 4000 and 7000 ppm and only after delivery of the last litter (PND 14-21) at 1000 ppm. Food consumption was decreased mainly during the lactation period for the final litter in the 4000 and 7000 ppm groups, and only during the last week of lactation for the 1000 ppm group. Water consumption during this period was highly variable, but tended be decreased in the 4000 and 7000 ppm groups. The body weight effects in treated $F_0$ dams were at least partly related to reduced fertility and fecundity. Five sets of litters were produced during the cohabitation period. In the first set of litters, fertility was reduced by 10% in the 7000 ppm group compared to controls. By the time of the final litter, significant reductions in the following reproductive parameters were observed in mice dosed at $\geq$4000 ppm: fertility index (fertile pairs/cohabiting pairs), the number of litters per pair, the number of live pups per litter, the percent of live pups, and live pup weight. Treatment had no effect on the cumulative days to litter. An increase in malformed pups was observed in treated groups. The proportion of litters with one or more pups with external abnormalities (domed heads, hematomas) was 7.9% in the control group and 10.5, 90 and 77.8% for the low- to high-dose groups. The authors attributed the decline in the proportion of pups with external abnormalities in the high-dose group (relative to the mid-dose group) to the decreased fertility, increased prenatal mortality and increased postnatal cannibalism of severely affected pups in that group. For the final $F_1$ litter, survival of pups during lactation was reduced in the $\geq$4000 ppm groups, but body weight was not affected in pups surviving to weaning. Reduced survival appeared to be related to the higher incidence of craniofacial abnormalities in mid- and high-dose groups.

In the $F_0$ crossover mating trials, results for the 7000 ppm dosed male group were not significantly different from those of the control group (Fail et al., 1998; NTP, 1992a). However, the dosed female group had significant reductions in the number of live pups per litter and the live pup weight. The offspring of dosed females showed significant increases in external malformations (90.9% of litters affected, compared to 12.5% for control offspring and 0 for offspring of dosed males mated to control females) and skeletal malformations (95% of pups within litters affected, compared to 40% for the control group and 38% for the dosed male group; percentage of litters affected was 100% compared with 83.3% for controls and 81.1% for the dosed male group). Skeletal malformations (abnormal ossification of cranial plates, abnormal or incomplete development of sternebrae) were qualitatively more severe in the offspring of dosed females, compared to the other two groups, in which developmental delay (delayed ossification) was the primary effect. The fact that the rate of the "malformations" described above in the control group (83% of litters; 40% of pups) was so high suggests that these might appropriately

be classified as variations rather than malformations. Offspring of dosed females, but not the other groups, had increases in facial and brain abnormalities: cleft palate, agnathia, agenesis of the cerebrum, abnormally-shaped cerebrum and enlarged cerebral ventricles. The results of the crossover trials indicate that reproductive effects of dimethylformamide are not likely caused by genotoxicity in sperm, but rather were mediated through the $F_0$ females. Exposure to 7000 ppm dimethylformamide had no significant effect on the length or stage frequency distribution of the estrous cycle, but did reduce the number of animals with normal cycle lengths by 20%.

At terminal necropsy of $F_0$ mice, dimethylformamide exposed mice showed no effect on body weight in males, but exposure at 7000 ppm caused a significant reduction in females (Fail et al., 1998; NTP, 1992a). In males, the absolute weights of the liver and right cauda epididymis were significantly increased at $\geq$1000 ppm, and the absolute weight of the prostate was significantly decreased at 7000 ppm. In females, the absolute and relative weights of the liver and the relative weight of the kidney/adrenals were significantly increased at $\geq$1000 ppm. The authors judged that histopathological findings (centrilobular hepatocellular hypertrophy) in two mid-dose females and two high-dose males with gross hepatic lesions were related to treatment. Dimethylformamide had no effect on reproductive histology or on epididymal sperm concentration, motility or morphology. The authors considered that a significant trend for a slight decrease in testicular spermatid concentration (significant at low and high doses) was not biologically relevant. Results for the first generation in the continuous breeding study indicate that 1000 ppm (185 mg/kg-day for males and 225 mg/kg-day for females) is considered a NOAEL and 4000 ppm (610 mg/kg-day for males and 788 for females) is considered a LOAEL for systemic (liver) effects. In the first generation study, the NOAEL for reproductive and developmental toxicity is 1000 ppm (225 mg/kg-day for females) and the LOAEL is 4000 ppm (788 mg/kg-day for females) for reductions in fertility, live pups, and pup birth weight, and increases in external and skeletal abnormalities, mediated through the $F_0$ females. These effects, however, were considered by the study authors to be due at least in part to the decreased female body weight and food and water consumption.

The assessment of fertility in $F_1$ mice was conducted using mice randomly selected at weaning (PND 21) from the four treatment groups (Fail et al., 1998; NTP, 1992a). Weanlings were dosed at 0, 1000, 4000 or 7000 ppm in drinking water beginning on PND 22. As above, doses were estimated as 0, 226, 947 and 1814 mg/kg-day for $F_1$ males and 0, 292, 1098 and 2054 for $F_1$ females. At PND 74, males and female nonsiblings were housed for mating for up to 7 days; 20 nonsibling pairs were established for the 0, 1000 and 4000 ppm groups. Since survival was affected at the highest dose, the group consisted of 15 pairs, some of which were siblings. After breeding, $F_1$ mice were housed singly through delivery of $F_2$ pups; the same reproductive endpoints were monitored as for the $F_0$ crossover trial. In addition, body weight and feed and water consumption were recorded on PND 74, 84 and 112; maternal body weights were recorded upon discovery of an $F_2$ litter. Vaginal smears were collected for 12 day following delivery. At scheduled necropsy at PND 119, $F_1$ adults were necropsied as described for $F_0$ mice, except that histopathology was not conducted on the prostate or seminal vesicles. Treatment with dimethylformamide reduced body weights of male and female $F_1$ mice exposed at $\geq$4000 ppm, but had no effect on feed consumption. Water consumption was increased for males at $\geq$4000 ppm on PND 84 and at 7000 ppm on PND 112. Exposure to dimethylformamide at 7000 ppm caused

reductions in the mating index. At ≥4000 ppm, there were reductions in fertility, the number of live $F_2$ pups per litter, pup body weight, adjusted (for litter size) pup body weight and proportion of live pups/litter and an increase in the average days to litter. At 1000 ppm, live pup weight and adjusted live pup weight were decreased. The proportion of litters with one or more externally malformed pups was 0, 27.7, 60 and 75%, respectively, for the control and low- to high-dose groups. Estrous cycle evaluations in control and 7000 ppm females showed that treated mice had longer cycles and were in either metestrus or diestrus longer than controls. At necropsy, $F_1$ body weights were reduced in both sexes at ≥4000 ppm. At ≥1000 ppm, absolute and relative liver weights were significantly increased in both sexes, and relative prostate weight was decreased in males. At ≥4000 ppm, absolute prostate weight was decreased in males, and relative kidney/adrenal weight was increased in females. Evaluations of livers with gross lesions from low- and high-dose group mice demonstrated dose-related centrilobular hepatocellular hypertrophy. Epididymal sperm concentrations were decreased at 7000 ppm. Average estrous cycle length was significantly increased in treated $F_1$ females, but treatment had no effect on sperm morphology. Examination of the skeleton of $F_1$ adults (5/sex/dose) revealed a malformation rate of 30, 20, 100 and 100% in the control and low- to high-dose groups. High incidence (30%) in controls could possibly suggest the increased skeletal malformations might be variants of normal. Malformations in the ≥4000 ppm groups included abnormal or incomplete ossification of cranial plates, abnormal cranial sutures, dysplasia of cranial bones and abnormal sternebrae. For the second generation study, the lowest drinking water concentration of dimethylformamide, 1000 ppm (equivalent to a dose of 226 mg/kg-day for males and 292 mg/kg-day for females) is a LOAEL for increased liver toxicity [increased organ weight; centrilobular hepatocellular hypertrophy (in two livers with gross lesions)] in $F_1$ adults (maternal toxicity) and for developmental toxicity (reduced live $F_2$ pup weight).

Gestational exposure studies of dimethylformamide have been conducted in rats, mice and rabbits. Saillenfait et al. (1997) evaluated developmental toxicity in groups of 22-24 pregnant Sprague-Dawley rats that were exposed to 0, 50, 100, 200 or 300 mg/kg of dimethylformamide (purity 99.9%) by gavage in distilled water on gestational days (GD) 6-20. Dams were observed daily for clinical signs; food consumption was measured every third day beginning on GD 6. Body weights were recorded on GD 0 and every third day beginning on GD 6. At termination on GD 21, dams were sacrificed and the number of implantation sites, resorptions (including early resorptions), and dead and live fetuses were recorded. Live fetuses were weighed, sexed, examined for external anomalies, and then examined either for visceral or skeletal anomalies. The study methods did not include observation of the dams for clinical signs or necropsy of the dams at termination. Treatment had no effect on survival of dams. Maternal body weight gain and food consumption were statistically significantly reduced in the ≥100 mg/kg-day groups in a dose-related manner; at 50, 100, 200 and 300 mg/kg-day for GD 6-21, mean maternal body weight gain was 3.9, 16.9, 33.8 and 40.3% less than controls, mean corrected maternal body weight gain (maternal body weight gain minus gravid uterine weight) was not different, 41.9, 79.1 and 90.7% less than controls and mean food consumption was 0.3, 15.0, 24.0 and 20.9% less than controls. Dose-related reductions in fetal body weight occurred for all (total) fetuses at ≥100 mg/kg-day, female fetuses at ≥100 mg/kg-day and male fetuses at ≥200 mg/kg-day; at 50, 100, 200 and 300 ppm, mean body weight of all fetuses per litter was 0.3, 4.3, 12.1 and 14.1% less than controls. Thus, the strongest effect was on the corrected maternal body weight gain, which

was affected much more severely than maternal food consumption. Effects on fetal body weight were less severe and appear to be secondary to effects on maternal body weight gain. Sporadic individual external and visceral malformations were observed in the litters of treated groups, but the incidences were not dose-related. Incidences of fetuses and litters with two skeletal variations, absent or incompletely ossified supraoccipital and sternebrae, were statistically significantly increased at ≥200 mg/kg-day. At 0, 50, 100, 200 and 300 ppm, for the supraoccipital skull variation, the incidences of affected fetuses were 2/122, 2/145, 9/140, 64/139 and 119/140 and of affected litters were 0/16, 2/20, 7/19, 16/19 and 20/20, and for the sternebral variation, the incidences of affected fetuses were 3/122, 13/145, 13/140, 15/139 and 32/140, and of affected litters were 2/16, 7/20, 7/19, 11/19, and 13/20. These skeletal variations reflect delayed ossification, and are consistent with body weight depression in the fetuses, which may be associated with the depression of food consumption and body weight gain in the dams (e.g., Fleeman et al., 2005; Hood, 2005). In this study, the maternal NOAEL was 50 mg/kg-day and the LOAEL was 100 mg/kg-day for reductions in food consumption and body weight gain. Whether the decreased food consumption was due to discomfort from the gavaged chemical, an affect on appetite, or other toxic effects of the chemical cannot be determined from the available data. The developmental NOAEL was 50 mg/kg-day and the LOAEL was 100 mg/kg-day for reduced fetal body weight gain. Thus, the apparent maternal NOAEL and LOAEL (for reduced food consumption and body weight gain) were the same as the developmental NOAEL and LOAEL.

Hellwig et al. (1991; BASF 1976a, b) conducted developmental toxicity studies in rats and mice exposed to dimethylformamide by gavage in water. The study authors stated that testing was conducted according to FDA (1966) guidelines. Groups of 19-23 pregnant Sprague-Dawley rats were given gavage doses of 166, 503 or 1510 mg/kg-day on GD 6-15; parallel "untreated' control groups (18-23 per group) were included. Dams were evaluated for mortality and clinical signs until GD 20, at which time Caesarian sections were performed. Other parameters analyzed at termination included gross pathology of dams, numbers of implantation and resorption sites (early, medium-term and late), numbers of living and diseased fetuses (per group and per dam), fetal weight, fetal length, fetal sex ratio and placental weight. Viable fetuses were examined for skeletal (2/3 total number) or soft tissue anomalies (1/3 total). Maternal effects included the death of one high-dose dam on GD 10, dose-related reductions in placental weights in all treated groups and dose-related reductions in body weight gain in mid- and high-dose dams (weight gain data not shown and food consumption not reported). The total number of live fetuses and the number of live fetuses per dam were significantly reduced in the high-dose group, primarily because of an increase in mid-term resorptions (>60%). The elevated incidence of early- and late-term resorptions was statistically significant in the mid-dose group, but not in the low-dose group. Dose-related increases in the number of resorptions (total and per implantation), in the total number of runts and in the percent of live fetuses with anomalies (primarily skeletal) were observed in the mid- and high-dose groups. Dose-related reductions compared to controls were observed in fetal weights and lengths in the mid- and high-dose groups. Unless specified otherwise in this description, the data do not appear to be reported on a per litter or per dam basis, and, thus, the reporting is not in accord with current guidelines. The 4% reduction of placental weight in the low-dose group, while not biologically significant by itself, represents the low end of the trend for reductions in placental, maternal and fetal weights observed at the higher doses. The low dose of 166 mg/kg-day is a minimal maternal reproductive LOAEL for reduced placental

weight. More severe maternal effects (more pronounced reductions in placental weight and reductions in body weight gain) and also developmental effects (e.g., resorptions and reduced fetal weight) were observed at 503 and 1510 mg/kg-day. The NOAEL and LOAEL for developmental effects are tentatively identified as 166 and 503 mg/kg-day, but the reliability is questionable because most of the statistical analyses did not appear to consider the litter as the unit for analysis and because controls were not gavage-treated with vehicle.

In their developmental study on mice, Hellwig et al. (1991) employed the same protocol used for rats with the following exceptions. Groups of 24 pregnant NMRI mice were given aqueous gavage doses of 182 or 548 mg/kg-day on GD 6-15; two "untreated" control groups (23 each) were included. Mouse pregnancies were terminated on GD 18. Treated dams showed no obvious effects on survival, body weight gain (not evaluated statistically and data not reported) or incidence of clinical signs. Treatment had no effect on placental weight, or the numbers of resorptions, implantations or implantations per dam. Gestational exposure resulted in reductions in fetal length in both groups and in fetal weight in the high-dose group. The number of anomalies was elevated in the high-dose group. In this study, the high dose of 548 mg/kg-day was a NOAEL for maternal toxicity. The low dose of 182 mg/kg-day may be considered a NOAEL for reduced fetal length in the absence of an effect on fetal weight, and 548 may be considered a LOAEL for reduced fetal body weight and length, and for increased anomalies. The reliability of the fetal NOAEL and LOAEL, however, is questionable since the statistical analyses generally did not appear to consider the litter as the unit for analysis, and because controls were not gavage-treated with vehicle.

Fritz and Giese (1990; Ciba-Geigy, 1979) evaluated the pre- and postnatal toxicity of dimethylformamide administered at doses of 0 or 750 mg/kg-day by gavage in water to pregnant Sprague-Dawley rats on GD 10-14. One set of controls and treated dams (10 per group) were sacrificed near term (GD 21) and the following endpoints were evaluated: number of litters, number of fetuses, mean fetal body weight, fetal mortality, gross malformations and skeletal anomalies. The offspring of another set (10 per group) were examined until postnatal day (PND 46). In addition to body weight, 13 randomly-selected pups from the treated group were evaluated on PND 5 for skeletal anomalies, and the surviving pups were evaluated on PND 32 for various neurological and behavioral parameters. Maternal endpoints, such as body weight, clinical signs, food consumption and gross pathology, were not reported for either set of animals, and fetal endpoints were not reported on a litter basis. In the prenatal study, exposure to dimethylformamide had no effect on the incidences of fetal mortality or gross malformations, but mean fetal weight was reduced by about 20% compared to the controls. In addition, the treated group exhibited significant increases compared to controls in the incidences of delayed ossification of certain bones: phalangeal nuclei (56.8 vs 8.5% for proximal phalanx V), calcaneus (93.7 vs 11.1%), frontoparietals (80.2 vs 0%) and supraoccipitals (100 vs 0%). A sex difference was noted in the frontoparietal effect; all treated male fetuses were affected, but only ~65% of females were affected. In addition, about 53% of treated fetuses exhibited fused sternebrae 4 and 5, an anomaly that was not observed in controls. In the postnatal study, gestational exposure to dimethylformamide had no effect on the direct pupil reflex, the startle response to a loud noise, or behavioral parameters (exploratory locomotion, cliff avoidance and negative geotaxis on a 45° slope). However, body weight gain was slightly reduced in the treated group and pups exhibited

a persistence of delayed ossification of the frontoparietal bones on PND 5. In this study, 750 mg/kg-day is a LOAEL for developmental effects (increases in delayed ossification and reduced fetal body weight gain). The delayed ossification effects occurred in such high percentages of fetuses that the lack of reporting on a litter basis does not impact the judgment that this dose is a LOAEL.

In an earlier study in rabbits, Merkle and Zeller (1980) treated groups of female Russian rabbits by gavage with dimethylformamide in aqueous solution on days 6-18 postinsemination. The dams were killed on day 28 postinsemination. The doses were 0, 46.4, 68.1 or 200 µL/kg-day. Using the density of dimethylformamide at 25 °C of 0.9445 g/mL or 0.9445 mg/µL, the doses are equal to 0, 43.8, 64.3 or 188.9 mg/kg-day. The highest dose was toxic to the dams, resulting in decreased food intake and weight gain. The rate of abortion in pregnant does was 1/22, 0/10, 0/16 and 3/11, in order of controls through highest dose. The number of implantations/dam and the number of live fetuses/dam in the mid-dose group were significantly ($p<0.01$) lower than control; however, no significant differences in these parameters were observed in the high-dose group. Statistically significant decreased mean fetal weight was observed at the high dose compared with controls ($36.3 \pm 3.19$, $35.3 \pm 3.77$, $36.0 \pm 4.97$ and $29.7 \pm 5.8$ g in the control, low-, mid-, and high-dose groups, respectively). There were statistically significant increases in the percentage of malformed live fetuses/litter (33.3%) and the percentage of litters with malformed fetuses (87.5%, seven of eight litters) in the high-dose group compared with controls (0% for both effects). These parameters were not significantly elevated in low- (1.1 and 10%) and mid- (3.6 and 13.3%) dose rabbits, although a few malformed fetuses were observed at the lower doses: one fetus with hydrocephalus in the low-dose group and three fetuses with hydrocephalus in 2 of 16 litters in the mid-dose group. At the high dose, there were six fetuses with hydrocephalus, one with abnormal position of the extremities, two with exophthalmos, three with simple eventration, seven with umbilical hernia and one with cleft palate. Litter incidences of specific effects were not reported. Thus, the high dose of 188.9 mg/kg-day was teratogenic, fetotoxic and maternotoxic in rabbits. The mid-dose of 64.3 mg/kg-day is considered a NOAEL in the absence of statistically significant effects on fetal weight and malformations at this dose.

*Inhalation Exposure*

The data set for inhalation toxicity of dimethylformamide includes subchronic toxicity studies in monkeys, rats, mice and other species (Hurtt et al., 1992; Craig et al., 1984; NTP, 1992b; Tanaka, 1971; Massmann, 1956b; Clayton et al., 1963), chronic toxicity studies in rats and mice (Malley et al., 1994), and developmental toxicity studies in rabbits and rats (Hellwig et al., 1991; Lewis et al., 1992; Kimmerle and Machemer, 1975; BASF, 1974).

Hurtt et al. (1992) whole-body exposed groups of cynomolgous monkeys (3/sex/group) to filtered room air or to dimethylformamide (purity >99%) at inhalation concentrations of 30, 100 or 500 ppm (90, 300 or 1500 mg/m$^3$) for 6 hours/day, 5 days/week for 13 weeks. The test atmospheres were primarily vapor; the aerosol concentration of the 500 ppm atmosphere was calculated to be less than 0.01% of the total mass of dimethylformamide. Since the lower concentrations were generated by diluting the 500 ppm atmosphere with filtered air, the aerosol

concentrations were likewise reduced. The study included satellite groups of two males per exposure concentration that were given a 13-week recovery period after the last exposure. Assignment of males to the exposure groups included pre-exposure assessment of sperm parameters so that group mean values were similar. Monkeys were observed twice daily for morbidity or mortality and once per week for clinical signs of toxicity. Body weights were measured prior to the first exposure, weekly thereafter, and at termination. Hematology and serum chemistry analyses were conducted on blood samples from all monkeys prior to and at the end of the first exposure, at the end of weeks 2, 4, 8 and 12, and at scheduled necropsy. Two monkeys/sex/group were placed in metabolism chairs to collect 6-hour samples for urinalysis on the same schedule. Sperm concentration, motility and morphology were analyzed in semen collected from males 3 times prior to the start of the study (baseline) and once a week thereafter. Menstrual cycles were monitored by daily vaginal smears beginning about 2 weeks after the first exposure. At scheduled necropsy, organ weights were measured for the liver, thyroid/parathyroid (paired), pituitary, heart, lungs, individual kidneys, testes and ovaries. More than forty tissues, including three sections of nasal epithelium, were evaluated for histopathology in all animals. Inhalation exposure to dimethylformamide had no effect on mortality, the incidence of clinical signs, body weight changes, hematology, serum chemistry, urinalysis, semen volume and sperm analyses, menstrual cycles, organ weights, gross necropsy findings or incidence of histopathological lesions. (Some lesions in lung, liver and lymph nodes were attributed to parasitic infection.) In this study, the highest exposure level, 500 ppm (1500 mg/m$^3$), was a NOAEL for cynomolgous monkeys exposed by inhalation to dimethylformamide.

Craig et al. (1984) exposed 10 F344 rats/sex or 10 B6C3F1 mice/sex to 0, 150, 300, 600 or 1200 ppm (0, 448, 897, 1794 or 3587 mg/m$^3$) of dimethylformamide for 6 hours/day, 5 days/week for 12 weeks. Animals were observed for clinical signs of toxicity, body weights were determined biweekly and complete gross necropsies were performed on all animals. Clinical chemistry analyses were performed on all animals that survived to terminal sacrifice. Histopathological evaluation was conducted on the lungs, heart, liver, thymus, spleen, pancreas, kidneys, urinary bladder, testes and nasal passages. The rats exposed to 1200 ppm showed few signs of overt toxicity, but their weight gain was significantly less than the control animals. The female rats exhibited an increase in serum AP, and one high-dose animal had high ALT and AST levels that may be indicative of acute hepatocellular injury. Gross necropsy findings included increased discoloration of the lungs in high-dose rats, but no lesions were found in the lungs or nasal turbinates in histological examination. The only treatment-related changes occurred in the liver. These changes consisted of pale, enlarged livers with an accentuated lobular pattern and/or prominent capsular blood vessels. Histopathology revealed that the livers of two rats that died during the study exhibited wide-spread collapse, necrosis, and accumulation of yellow-brown pigments in Kupffer cells, macrophages and hepatocytes. The livers of the high-dose females were found to contain areas of collapse near the central veins with occasional fibrosis and yellow-brown pigment, and large variations in nuclear size and cytoplasmic characteristics. The livers of the females exposed to 300 or 600 ppm of dimethylformamide showed variation in nuclear size and cytoplasmic characteristics that were similar to the higher doses, but to a lesser extent. No changes were seen in the livers of rats exposed to 150 ppm of dimethylformamide. Livers from the male rats exhibited the same changes as those described for the females except

that there was no collapse or fibrosis. A LOAEL of 300 ppm (897 mg/m$^3$) and a NOAEL of 150 ppm (448 mg/m$^3$) is identified for these hepatic cell changes.

The mice exposed to dimethylformamide exhibited no clinical signs of toxicity, no changes in body weight gain and no hematologic or clinical chemistry changes attributable to treatment (Craig et al., 1984). However, eight high-dose mice and three mice exposed to 600 ppm dimethylformamide died or were sacrificed moribund during the study. The livers from the treated mice in all groups revealed collapse, necrosis and yellow-brown pigment. Hepatic cytomegaly was observed in all exposed groups and the incidence and severity of this lesion were dose-related. No effects were reported in the respiratory tract. These results indicate a LOAEL of 150 ppm (448 mg/m$^3$) for dimethylformamide in mice; a NOAEL was not identified.

NTP conducted subchronic inhalation toxicity studies of dimethylformamide in rats and mice (Lynch et al., 2003; NTP, 1992b). Groups of F344/N rats (30/sex/group) were whole-body exposed to dimethylformamide (purity >99%) vapor at target concentrations of 0 (chamber control), 50, 100, 200, 400 or 800 ppm (0, 150, 300, 600, 1200 or 2400 mg/m$^3$) for 6 hours (plus $T_{90}$)/day, 5 days/week for 13 weeks. Each group was equally divided into three subgroups, a base study group, a cardiac physiology group and a urinalysis/renal function group. Rats were observed twice daily for mortality and moribundity; body weights and clinical signs were measured weekly and at necropsy. Comprehensive hematology and clinical chemistry studies were performed on cardiovascular group rats at 4 and 23 days and on base-study rats at 13 weeks. Reproductive system evaluations were performed on all rats exposed to 0, 50, 200 or 800 ppm; sperm counts, morphology and motility were assessed at necropsy, and vaginal cytology was evaluated during the 2 weeks preceding necropsy. At 13 weeks, comprehensive urinalysis was conducted on five male and five female rats in the 0, 50, 200 and 800 ppm groups, and the kidneys of these rats were evaluated for histopathology. Within 24 hours after the final exposure, the cardiovascular groups were evaluated for blood pressure and electrocardiograms, and the hearts were examined for histopathology. At termination, complete necropsies were performed on rats in the base-study and renal function groups. Organ weights were recorded for the liver, thymus, both kidneys, both testicles, heart and lungs. Gross lesions and more than thirty tissues, including nasal turbinates, were evaluated for histopathology in all control and high-dose rats in the base study; liver histopathology was assessed in all lower dose groups as well.

Dimethylformamide exposure had no effect on survival in the rats (Lynch et al., 2003; NTP, 1992b). Concentration-dependent depression in body weight gain occurred over the course of the study in both sexes at 400 ppm (6-11%) and 800 ppm (20-22%). Hematology evaluation of the males showed increases in hematocrit and hemoglobin concentration at 800 ppm and erythrocyte counts at $\geq$400 ppm at 24 and 91 days; the same parameters were elevated in the 800 ppm females at 4, 24 and 91 days. These findings were characterized as evidence of mild hemoconcentration consistent with a mild dehydration, and not attributed to dimethylformamide exposure. Reasons for the mild dehydration were not presented, and water and food consumption were not monitored. Platelet counts were elevated in males at $\geq$100 ppm at 24 and 91 days; in females, platelets were elevated in the $\geq$200 ppm groups at 24 days, but the values at 91 days were not significantly different from controls. Serum cholesterol was elevated in both sexes at $\geq$50 ppm at 4, 24 and 91 days; at 50, 100, 200, 400 and 800 ppm on day 91, increases were 13.3,

22.9, 18.1, 18.1 and 61.4% higher than controls in males, and 11.3, 33.0, 18.6, 41.2 and 40.2% higher than controls in females. Serum ALT was increased in all males at ≥400 ppm at all time points. ALT was elevated in females at 800 ppm at all time points and at ≥100 ppm on day 24. Total bile acids were increased in males at ≥400 ppm at all time points and in females at 800 ppm on day 4, at ≥200 ppm on day 24 and at ≥400 ppm on day 91. Isocitric dehydrogenase activity was increased in both sexes at 800 ppm at all time points. Sorbitol dehydrogenase activity was increased in both sexes at ≥200 ppm at all time points; at 50, 100, 200, 400 and 800 ppm on day 91, increases were 17.1, 17.1, 100, 169 and 549% higher than controls in males, and 0, 11.5, 53.8, 84.6 and 558% higher than controls in females. Concentrations of total protein and albumin were decreased in males at ≥200 ppm on day 4, but were elevated in the ≥400 ppm groups on day 24 and the 800 ppm group on day 91. In females, total protein and albumin were decreased at 800 ppm on day 4, at 400 ppm on day 24 and at ≥200 ppm on day 91. Absolute lung weights were significantly decreased in males and females exposed at ≥50 ppm; relative lung weights were significantly lower in all exposed groups except for males at 400 ppm and females at 800 ppm. Relative liver weights were increased in both sexes at ≥50 ppm; absolute liver weights were increased in females at ≥50 ppm and males at ≥200 ppm. Relative kidney weights were increased in males at ≥100 ppm and females at 800 ppm. There were no treatment-related changes in urinalysis indices. Exposure had no effect on heart rate or blood pressure. Eight exposed rats had qualitatively abnormal electocardiographic waveforms, but there was no consistent relationship to concentration. Absolute heart weights were reduced in males and females at ≥400 ppm, but this was related to the reduced body weight. Treatment had no adverse effect on sperm parameters or testicular/epididymal weight or histology. There were no significant group differences in estrous cycle length, although alterations (prolonged diestrus) occurred at 800 ppm: undefinable or longer than 12 days in 7/10 animals and slightly longer than controls in 3/10 animals (5.3 vs 5.0 days). No gross pathology was observed in any organ or tissue and histopathological changes were found only in the liver. Liver lesions occurred at ≥400 ppm in both sexes and included hepatocyte necrosis in 10/10 and 10/10 males and 8/10 and 10/10 females at 400 and 800 ppm, and pigment in macrophages in 10/10 males and 10/10 females at 800 ppm; incidences of both lesions were 0/10 in all lower dose and control groups. As summarized above, the liver was the most sensitive target as shown by effects that included increased serum cholesterol and relative liver weight at ≥50 ppm, increased serum sorbitol dehydrogenase at ≥200 ppm and increased serum ALT and liver lesions (hepatocyte necrosis) at ≥400 ppm. The toxicological significance of the serum cholesterol changes is unclear because the increases were small (particularly at the lower concentrations) and not clearly dose-related, and information on normal ranges in F344/N rats of comparable age (19 weeks) was not located. Based on increased serum sorbitol dehydrogenase at 200 ppm and supporting histological evidence of liver cell necrosis at 400 ppm, the LOAEL is judged to be 200 ppm (600 mg/m$^3$). The NOAEL is 100 ppm (300 mg/m$^3$), the highest concentration causing increased liver weight in the absence of the serum enzyme indication of hepatocellular damage.

The subchronic inhalation study in B6C3F$_1$ mice (Lynch et al., 2003; NTP, 1992b) used the same experimental design as for the rats, including exposure to 0, 50, 100, 200, 400 or 800 ppm dimethylformamide for 6 hours/day, 5 days/week for 13 weeks, except for smaller numbers of animals (10/sex/group), minor differences in the tissues selected for histopathology and lack of hematology, clinical chemistry, urinalysis and satellite groups for cardiovascular and renal

studies. There were no exposure-related effects on survival, body weight gain or incidence of clinical signs in mice. Treatment had no effect on testicular/epididymal weights or sperm parameters. There were no significant group differences in estrous cycle length, although there was a significant trend (p=0.035) toward an increase in estrous cycle length (4.15, 4.05, 4.55 and 4.80 days at 0, 50, 200 and 800 ppm; data for 400 ppm were not reported). Relative and/or absolute kidney and lung weights were increased in all exposed female groups, but not in males. Relative liver weights were increased in both sexes at ≥50 ppm; absolute liver weights were increased in males at ≥200 ppm and in females at ≥50 ppm. Necropsy showed a gross liver lesion that may have been exposure-related; tan foci were noted in the liver of one male each in the 400 and 800 ppm groups. Histological changes were also limited to the liver, consisting of minimal to mild centrilobular hepatocellular hypertrophy in males at ≥50 ppm and females at ≥100 ppm. Incidences of this effect at 0, 50, 100, 200, 400 and 800 ppm were 0/10, 4/10, 9/10, 10/10, 10/10 and 10/10 in males, and 0/10, 0/10, 10/10, 10/10, 10/10 and 10/10 in females. The lowest exposure level of 50 ppm (150 mg/m$^3$) is classified as a LOAEL based on increases in liver weight and histological changes (centrilobular hepatocellular hypertrophy) supported by possible gross liver pathology at 400 ppm.

Senoh et al. (2003) evaluated the subchronic inhalation toxicity of dimethylformamide in rats and mice. Groups of 10 male and 10 female F344/DuCrj rats were exposed to dimethylformamide (>99.8% pure) in target concentrations of 0, 50, 100, 200, 400 or 800 ppm (0, 150, 300, 600, 1200 or 2400 mg/m$^3$) for 6 hours/day, 5 days/week for 13 weeks. Deviations of mean observed concentrations from the target levels were <3.5%. The study conformed to OECD guidelines for a 90-day inhalation toxicity study. Animals were assessed daily for clinical signs and mortality and weekly for body weight and food consumption. Comprehensive hematological, blood biochemical, urinary, gross pathological, organ weight and histological evaluations were performed on all surviving animals at the end of the 13-week exposure period. Statistically significant effects included reduced body weight gain in both sexes at ≥400 ppm, reduced food consumption in both sexes at 800 ppm, increased serum total cholesterol in males at ≥50 ppm (58.9-142.9% higher than controls) and females at ≥200 ppm (64.2-107.4% higher) and increased serum phospholipids in males at ≥50 ppm (48.6-70.1% higher than controls) and females at ≥100 ppm (34.8-64.5% higher). Other statistically significant changes included increases in triglycerides in females at ≥200 ppm and males at 800 ppm, total bilirubin in females at ≥400 ppm and males at 800 ppm, serum ALT in females at ≥400 ppm and males at 800 ppm and γ-GTP and ALP in females at ≥400 ppm; no effects on serum AST and LDH were observed in either sex. Relative liver weight was statistically significantly increased in males at ≥100 ppm and females at ≥200 ppm. Absolute liver weights tended to increase in both sexes but were not clearly dose-related (significantly increased in males at 50-200 ppm and females at 50-100 ppm and 800 ppm). Histopathological changes were limited to the liver and included significantly increased incidences of single-cell necrosis of hepatocytes in both sexes at ≥200 ppm (80-100% compared to 0% at 0-100 ppm) and centrilobular hepatocellular hypertrophy at ≥400 ppm (80-100% compared to 0% at 0-200 ppm). As summarized above, the most sensitive effects were increased serum cholesterol and phospholipids at 50 ppm, increased relative liver weight at 100 ppm and liver lesions (single-cell necrosis) at 200 ppm. The toxicological significance of the changes in serum cholesterol and phospholipids is unclear because the increases were not pronounced (except for cholesterol in high-dose males), standard deviations were not reported and

information on normal ranges in F344/DuCrj rats could not be located. The LOAEL is 200 ppm (600 mg/m$^3$) based on liver histopathology (single-cell necrosis) and the NOAEL is 100 ppm (300 mg/m$^3$), the highest level causing increased liver weight in the absence of histopathology.

Groups of 10 male and 10 female Crj:BDF$_1$ mice were exposed to 0, 50, 100, 200, 400 or 800 ppm (0, 150, 300, 600, 1200 or 2400 mg/m$^3$) dimethylformamide for 6 hours/day, 5 days/week for 13 weeks (Senoh et al., 2003). The experimental design is the same as in the rat part of this study summarized above. Statistically significant effects included decreased body weight gain in males at $\geq$50 ppm and food consumption in males at 800 ppm; there were no effects on these endpoints in females. Blood biochemical changes occurred in both sexes including statistically significant increases in total cholesterol in females at $\geq$50 ppm (26.4-38.9% higher than controls) and males at 100 and 400 ppm (18.8 and 27.5% higher; non-significantly increased at 200 and 800 ppm), serum ALT in females at $\geq$200 ppm and males at 800 ppm, serum ALP in males at 800 ppm, and LDH and blood urea nitrogen in females at 800 ppm. Relative liver weight was significantly increased in males at $\geq$50 ppm, absolute liver weight tended to increase in males but was not clearly dose-related (significantly increased at 100-400 ppm) and liver weight was not significantly affected in females. Histopathological changes were limited to the liver and included significantly increased incidences of centrilobular hepatocellular hypertrophy in males at $\geq$50 ppm (40% at 50 ppm and 100% at 100-800 ppm compared to 0% in controls) and females at 800 ppm (70% compared to 0% at 0-400 ppm), focal necrosis in females at 100-400 ppm (50-70% compared to 0% in controls and 10% at 50 and 800 ppm) and single-cell necrosis in males at 800 ppm (60% compared to 0% at 0-200 ppm and 10% at 400 ppm) and females at 800 ppm (50% compared to 0% at 0-400 ppm). As summarized above, the most sensitive effects in this study were increased serum cholesterol, increased relative liver weight and centrilobular hypertrophy at 50 ppm and focal necrosis at 100 ppm. The toxicological significance of the serum cholesterol changes is unclear because the increases were small, standard deviations were not reported and information on normal ranges in Crj:BDF$_1$ mice could not be located. The lowest exposure level, 50 ppm (150 mg/m$^3$), is the LOAEL based on increases in liver weight and hepatocellular hypertrophy at 50 ppm and supporting evidence of focal necrosis at 100 ppm.

Malley et al. (1994) evaluated chronic inhalation toxicity and oncogenicity in rats and mice. Groups of CD rats (78/sex/group) were whole-body exposed to humidified air (control) or atmospheres containing 25, 100 or 400 ppm (75, 300 or 1200 mg/m$^3$) of dimethylformamide vapor for 6 hours/day, 5 days/week (excluding holidays) for 2 years. Rats were examined twice daily to detect mortality, morbidity and abnormal behavior and appearance. Rats were weighed and examined for clinical signs once per week for the first 3 months and once every other week thereafter. Ophthalmological examinations were conducted prior to the first exposure and immediately prior to scheduled sacrifice. Hematology, clinical chemistry and urinalysis were conducted at 3, 6, 12, 18 and 24 months on rats randomly selected prior to the first exposure: one set of 10 per sex per group examined at the first three time points and sacrificed at 12 months and a second set examined at 18 and 24 months. All rats were examined by gross necropsy. At scheduled necropsies, organ weights were recorded for the lungs, brain, liver, kidneys, adrenals, ovaries and testes. For all animals, gross lesions and more than 40 tissues were processed for histopathology. All tissues were examined microscopically for the control and 400 ppm groups

and for rats found dead or euthanized *in extremis*. The nose, lungs, liver, kidneys and all gross lesions were examined microscopically for the 25 and 100 ppm groups; the uterus was also examined in these groups because of the incidence of endometrial stromal polyps in the 400 ppm females. Cell proliferation in the liver, as measured by hepatocyte labeling following intraperitoneal injection with 5-bromo-2'-deoxyuridine (BrdU), was evaluated in rats (5/sex/group) after 2 weeks, 3 months and 12 months of testing; all livers were examined microscopically, but the control and 400 ppm livers were also evaluated immunohistochemically. The estrous cycle was monitored in control and 400 ppm females by vaginal smears taken on days 107-131.

Chronic inhalation exposure to dimethylformamide had no effect on survival, ophthalmoscopic findings, hematology parameters or estrous cycle in rats (Malley et al., 1994). Mean body weight gains were significantly reduced in males at $\geq$100 ppm and in females at 400 ppm. Serum levels of sorbitol dehydrogenase activity were significantly increased in both sexes exposed at $\geq$100 ppm for 3 or 12 months, and in males exposed at $\geq$100 ppm and females exposed at $\geq$25 ppm for 18 months; significant elevations in all treated male groups at 24 months were attributed to an unusually low mean value for control males. Urinalysis results were not reported. Relative liver weights were significantly increased in both sexes exposed at $\geq$100 ppm for 12 months or at 400 ppm for 24 months; significant elevations were also noted in females euthanized for the hepatic cell proliferation studies on day 19 (all exposed groups) and on day 95 (400 ppm group only). The authors did not consider the elevation in 25 ppm females on day 19 to be toxicologically significant because of its transience and because it was not associated with histopathology. No significant increase in the incidence of gross lesions was observed in rats exposed for 12 or 24 months. However, the incidence and severity of liver histopathology increased during the course of the study. At 12 months, the incidence of centrilobular hepatocellular hypertrophy was elevated in males at 400 ppm and in females at $\geq$100 ppm; in addition, the incidences of single-cell necrosis, accumulation of lipofuscin/hemosiderin, and clear cell foci were increased in both sexes exposed at 400 ppm. At 24 months, the incidences of centrilobular hepatocellular hypertrophy and accumulation of lipofuscin/hemosiderin were elevated in both sexes at $\geq$100 ppm; single cell necrosis was increased in males at 400 ppm and in females at $\geq$100 ppm; clear cell foci were increased at $\geq$100 ppm in males, and clear and eosinophilic foci were increased at 400 ppm in females. Hepatic cell proliferation in males at 400 ppm was slightly elevated, but no statistically significant difference was observed for treated rats of either sex compared to controls. The incidence of endometrial stromal polyps was elevated in rats at 400 ppm compared to controls (1.7, 5.1, 3.4 and 14.8% for the control and 25, 100 and 400 ppm groups, respectively). Since the incidence of the lesion was considered highly variable, and the incidence at 400 ppm was within the historical control range for the laboratory (2.0-15.0%), the authors considered the lesion to be a chance variation and not an oncogenic effect of treatment. No treatment-related lesions were observed in any other tissue, including the nose or respiratory tract. In this study, 25 ppm (75 mg/m$^3$) was a NOAEL and 100 ppm (300 mg/m$^3$) was a LOAEL for hepatic effects (increases in relative organ weight, histopathology and serum sorbitol dehydrogenase activity) in male and female rats exposed for 24 months.

The protocol for CD-1 mice in the chronic inhalation study of Malley et al. (1994) was similar to that for rats with the following exceptions. Groups of mice (78/sex/group) were

exposed to dimethylformamide at the same concentrations (0, 25, 100 and 400 ppm or 0, 75, 300 and 1200 mg/m$^3$) for 6 hours/day, 5 days/week for 18 months (study termination). Clinical evaluations of mice (10/sex/group) at 3, 6, 12 and 18 months only included hematology and not clinical chemistry or urinalysis. Adrenals were not weighed at scheduled necropsy. The battery of tissues processed for histopathology included the gall bladder, but excluded the prostate. Chronic inhalation exposure to dimethylformamide had no effect on survival, ophthalmoscopic findings, hematology parameters or estrous cycle in mice. Body weight gain was elevated in males exposed at 400 ppm and in females exposed at $\geq$100 ppm during the first year of the study (results for the period between 12 and 18 months were not reported). Progressive hepatic effects were observed during the course of the study. Absolute and relative liver weights were significantly increased in both sexes exposed to 400 ppm at hepatocyte proliferation terminations on days 19 and 95; at the hepatocyte proliferation termination on day 363, the same weight increases at 400 ppm were significant in males but slight in females. At 18 months, absolute and relative liver weights were significantly increased in males at $\geq$100 ppm and females at 400 ppm.

Gross necropsies revealed a higher incidence of enlarged livers and liver deformities (not specified) in males exposed at 400 ppm (Malley et al., 1994). Dose-related increases in liver histopathology were observed in all treated groups of males and females. Increases in centrilobular hepatocellular hypertrophy and single-cell necrosis were observed in both sexes exposed at $\geq$25 ppm. Hepatic Kupffer cell hyperplasia with accumulation of lipofuscin and hemosiderin and an increase in the incidence of inflammatory cells in the liver were observed in males at $\geq$25 ppm and in females at $\geq$100 ppm. Mixed hepatic foci were observed in male mice exposed at $\geq$100 ppm. The authors considered that 'secondary changes' observed in livers from mice exposed at $\geq$100 ppm were adaptive or indicative of repair activity: biliary hyperplasia, increased mitotic figures and multinucleate hepatocytes. The hepatocyte proliferation assays did not detect any differences between treated and control mice. No treatment-related lesions were observed in any other tissue, including the nose or respiratory tract. Treatment with dimethylformamide did not significantly increase the incidence of any specific tumor type. The authors reported that the incidence of total primary tumors and total benign tumors was significantly increased in males exposed at 400 ppm because of higher combined numbers for lung, liver and harderian gland tumors. The authors discounted these as related to treatment because the individual increased incidences were not statistically significant and because they are reported to have a high spontaneous incidence in male mice. However, the authors did not provide any quantitative data for the tumors in this mouse study or the historical control incidences. In this study, the low dose of 25 ppm was a LOAEL (75 mg/m$^3$) for hepatic effects in mice: increases in centrilobular hepatocellular hypertrophy and single-cell necrosis in males and females and Kupffer cell hyperplasia with accumulation of pigment in males. A NOAEL was not identified.

Tanaka (1971) studied the effects of inhalation exposure to dimethylformamide on female Sprague-Dawley rats of varying ages. Measurement of liver enzyme activities in serum and liver histopathology were conducted on 3-, 4-, 5-, 8-, and 12-week-old rats exposed to 200 ppm of dimethylformamide (598 mg/m$^3$) 8 hours/day 7 days/week for 4 weeks. Serum ALT and AST activities were increased in the 3- and 4-week-old rats, but not in the rats that were 5 weeks or older. Serum AP activity was increased in rats that were up to 5 weeks of age. Histopathological

changes were observed primarily in the livers of the younger animals, including degeneration, cloudy swelling of liver cells, and isolated cases of fatty degeneration primarily in the central zone. This study identifies a LOAEL of 200 ppm (598 mg/m$^3$) for liver damage. Tanaka (1971) also exposed groups of 3-week-old female rats to 200 ppm of dimethylformamide for either 1 or 8 hours/day every day for 4 weeks. The rats were sacrificed after 1, 2 or 4 weeks and liver function and pathology were evaluated. Serum AST and ALT were elevated in both groups of animals. The liver damage observed in the exposed animals was qualitatively similar across both groups (degeneration with evidence of regeneration after 2 and 4 weeks of exposure), but the animals exposed to dimethylformamide for 8 hours/day showed more severe lesions.

Massmann (1956b) exposed groups of 2 cats and 16 rats to either 100, 230, or 450 ppm (299, 688 or 1345 mg/m$^3$) of dimethylformamide 8 hours/day, 6 days/week for 120 days. Clinical signs of toxicity, body weight, hematological parameters and liver function were measured, as well as the electrocardiogram (in cats only). No overt signs of toxicity were noted in the rats, but the cats ate less and lost weight. No hematological changes were noted in either species, and the liver function tests were normal. Necropsy revealed an "irregular incidence" of liver necrosis in the rats, but the cats exhibited only fatty degeneration without necrosis. Other changes noted in the rats included bronchopneumonia, hyperemia of the brain, cloudy swelling of the uriniferous tubules of the kidney and iron deposits in the spleen. Data on incidence of these effects are not included in the report, but it is implied that liver changes were noted in the cats at dimethyl-formamide concentrations of 100 ppm or more, while the rats were not adversely affected at dimethylformamide concentrations below 450 ppm. Therefore, the NOAEL in rats is 230 ppm (688 mg/m$^3$).

Clayton et al. (1963) reported that rats exposed to 91 ppm (272 mg/m$^3$) dimethylformamide 6 hours/day for 10 days had increased relative liver weight. Clayton et al. (1963) also exposed mice (11 females), rats (10/sex), guinea pigs (10 males), rabbits (2/sex), and dogs (4 males) to 23 ppm of dimethylformamide for 5.5 hours/day, followed by 426 ppm for 0.5 hours/day for 58 weekdays. This exposure regimen was designed to simulate peak exposures that occur in plant operations. No adverse clinical signs were seen in any species except dogs, where one of the four animals had a decrease in systolic blood pressure. This dog also exhibited degenerative myocardial changes at necropsy. Increased liver weights were seen in all species except the guinea pig, but the difference was statistically significant only in mice. Plasma cholesterol levels were increased in the rats, rabbits, and in the single dog that demonstrated cardiovascular changes. Rat liver fat content was also increased. A LOAEL of 56.6 ppm (172 mg/m$^3$) is calculated as a TWA concentration for a 6-hour exposure.

Hellwig et al. (1991) evaluated the developmental toxicity of dimethylformamide in rabbits and rats exposed by inhalation. Groups of 15 artificially inseminated Himalayan rabbits were exposed (whole-body) to air or dimethylformamide vapor at concentrations of 50, 150 or 450 ppm (150, 450 or 1350 mg/m$^3$) for 6 hours/day on GD 7-19. Dams were observed until GD 29 and then subjected to caesarean section and necropsied. The following endpoints were evaluated: maternal body weight, clinical signs, food consumption, gross pathology, corpora lutea, uterine and placental weight, conception rate, living and resorbed implantations, pre- and postimplantation loss, live fetuses, fetal weight and fetal length. Live fetuses were evaluated for

external, soft-tissue or skeletal findings (retardations, variations or anomalies). Exposure to dimethylformamide vapor had no effect on mortality, the incidence of clinical signs, necropsy findings, uterine weights, or conception rate in dams. Maternal body weight gain was reduced in dams at 150 ppm, and body weight loss occurred in the 450 ppm dams during the period of exposure (GD 7-19); maternal body weight gain for the entire study (GD 0-29) was lower in treated groups than in controls, but the difference was not statistically significant and did not show a clear concentration-response. Exposure at 450 ppm resulted in significant reductions in fetal weights, and significantly increased total incidences of external malformations (mostly hernia umbilicalis) and skeletal abnormalities (sternal anomalies and variations). In the 150 ppm group, one hernia umbilicalis and a slight increase, of borderline significance, in the incidence of fused sternebrae was observed ($p>0.053$ in Fisher Exact test conducted for this review). In this study, the maternal NOAEL was 50 ppm (150 mg/m$^3$) and the LOAEL was 150 ppm (450 mg/m$^3$) for reduced body weight gain (during exposure). The developmental NOAEL was 50 ppm (150 mg/m$^3$) and the LOAEL was 150 ppm (450 mg/m$^3$) for minimal increases in the incidences of certain anomalies (hernia umbilicalis and fused sternebrae) that were statistically significant at the highest concentration.

Hellwig et al. (1991) evaluated the inhalation developmental toxicity in rats in two separate experiments using different discontinuous exposure protocols. In each experiment, groups of 30 pregnant Sprague-Dawley rats were whole-body exposed to air or 287 ppm (860 mg/m$^3$) of dimethylformamide vapor for 6 hours/day. In experiment I, exposures were on GD 0-1, 4-8, 11-15 and 18-19; in experiment II, rats were exposed on GD 0-3, 6-10 and 11-18. Randomly selected dams from each group were permitted to litter and raise offspring (satellite group). The following parameters were recorded: clinical signs, mortality, gross pathology, uterine weights, conception rates, implantations (total, live and dead), resorptions (early, medium-term and late), pre- and postimplantation loss, placental weight, and sex, length and weight of live fetuses. Live fetuses were examined for external anomalies and then for soft tissue (1/3) or skeletal anomalies (2/3). Satellite groups were evaluated for conception rate, implantations and resorptions, litter size, pup weight gain, pup mortality, viability and lactation index; on postnatal day 20, pups were subjected to a gross necropsy, including assessments of organ weights and head structure. Diseased pups were evaluated for skeletal anomalies. Results were similar in the two experiments. Under either protocol, exposure at 287 ppm for 6 hours/day reduced maternal weight gain by about half. The number of early resorptions and dead implants was elevated in both experiments; the increase in dead implants was statistically significant in experiment I. In both experiments, there were significant reductions in fetal weight, fetal length and placental weights in the groups exposed at 287 ppm. Exposed groups showed increases in the incidence of skeletal variations (sternal aplasia and displacement) and retardations (not specified) compared to the controls. Exposure had no effect on pups in the satellite group. The single exposure level of 287 ppm (860 mg/m$^3$) is a LOAEL for maternal toxicity (reduced maternal weight gain and reduced placental weight) and developmental toxicity (reduced fetal weight and increases in skeletal variations).

Lewis et al. (1992) evaluated developmental toxicity in groups of 21 pregnant CD rats exposed to air or to 30 or 300 ppm (90 or 900 mg/m$^3$) of dimethylformamide vapor by whole-body inhalation for 6 hours/day on GD 6-15. Dams were evaluated daily for clinical signs,

weighed on GD 0, 6-15 and 21, and given a gross pathological examination on GD 21. The following endpoints were examined: number of corpora lutea, number and position of live, dead and resorbed fetuses, and fetal weight, length, sex, external malformations (all), visceral alterations (2/3) and skeletal changes (1/3). Exposure had no effect on survival or the incidence of clinical signs or necropsy findings in dams. Body weight gain was reduced during GD 6-15 in the dams exposed at 300 ppm. Fetal weight was also reduced in the 300-ppm group compared to controls. There were no dose-related malformations. In this study, 30 ppm (90 mg/m$^3$) was a NOAEL for both maternal and developmental toxicity and 300 ppm (900 mg/m$^3$) was a LOAEL for reduced body weight gain in dams and reduced fetal body weight.

Kimmerle and Machemer (1975) reported that exposure of 22 or 23 female rats to 18 or 172 ppm (54 or 514 mg/m$^3$) of dimethylformamide 6 hours/day on gestation days 6-15 produced no evidence of maternal toxicity or fetal malformations. However, fetal body weight was significantly reduced in the high-dose group as compared with the controls, suggesting a LOAEL at 172 ppm (514 mg/m$^3$) and a NOAEL at 18 ppm (54 mg/m$^3$).

In an unpublished experiment conducted by BASF (1974), pregnant rats were exposed to either 220 or 520 ppm (660 or 1554 mg/m$^3$) of dimethylformamide on gestation days 4-8. A significant reduction in maternal weight gain was observed at 520 ppm, and a reduction in fetal weight and length was observed in both treated groups. The mean number of live fetuses was reduced in animals exposed to 520 ppm. The authors report that there was "an increased number of retardations and variations" found in the offspring of animals exposed to 520 ppm of dimethylformamide, but these effects were not evident in the data presented. This study identifies a LOAEL for a reduction in fetal weight and length at 220 ppm (660 mg/m$^3$).

**Other Studies**

*Biochemical Changes*

In a study by Savolainen (1981), groups of 10 male Wistar rats were exposed to dimethylformamide in the drinking water at concentrations of 0, 1.4, 6.8 or 13.7 mmol/L (0, 102.3, 497.0 or 1001 mg/L); five rats/group were killed after 2 and 7 weeks, and determinations of brain enzyme activities were made. Based on body weight and water consumption data, the rats consumed total doses of dimethylformamide of 0, 1.5, 7.5 or 14.4 mmol/kg (0, 7.8, 47.0 or 75.2 mg/kg-day) after 2 weeks and 0, 4.7, 24.0 or 43.9 mmol/kg (0, 7.0, 35.8 or 65.5 mg/kg-day) after 7 weeks. No effects were observed on body weight gain or behavior. High-dose rats had significantly ($p<0.05$) decreased levels of cerebral glutathione at 2 and 7 weeks. A dose-related decrease in the activity of cerebral succinate dehydrogenase occurred that was significantly lower than control in the mid- and high-dose rats at 2 weeks; the activity remained depressed at 7 weeks. Azoreductase activity was decreased significantly at all dose levels at 7 weeks, while RNA content of the cerebrum was increased significantly at the high dose after 7 weeks. Biochemical changes in the glial cells included significantly increased acid proteinase in the high-dose group at 2 and 7 weeks, significantly dose-related increased activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase at 2 weeks, and significantly decreased glial cell succinate dehydrogenase at all levels after 7 weeks. These biochemical changes were discussed in relation

to the inhibitory action of formic acid, a minor metabolite of dimethylformamide, on mitochondrial cytochrome oxidase; the investigators concluded that dimethylformamide may affect glial cells by inhibiting mitochondrial respiration. This study found biochemical changes in the brain occurring primarily at doses of 36 mg/kg-day or above. Only a few minor changes were seen at 7 mg/kg-day. None of the endpoints monitored are clearly indicative of an adverse effect.

In a similar study of the effects of dimethylformamide on the drug-metabolizing enzymes of the liver and kidney, groups of 10 male Wistar rats received the compound in the drinking water at 0, 100, 500 or 1000 mg/L (Elovaara et al., 1983). By analogy to the Savolainen (1981) study conducted in the same laboratory, corresponding doses were approximately 0, 7, 36 or 66 mg/kg-day. Five rats/group were killed after 2 and 7 weeks, and livers and kidneys were removed, weighed and homogenized. No effect on body weight gain or kidney weight was observed; however, there was a dose-related significant increase in relative liver weight after 2 weeks (statistically significant in the 36 and 66 mg/kg-day groups), which became more pronounced at 7 weeks (statistically significant in all treated groups). In the liver, no treatment-related effects on cytochrome P450 content or NADPH-cytochrome c reductase activity occurred, but glutathione levels, 7-ethoxycoumarin o-deethylase activity, and most markedly, UDP glucuronyl transferase activity all increased in a time- and dose-related manner (statistically significant differences from control primarily at 36 and 66 mg/kg-day). Although incubation of dimethylformamide with rat liver microsomes did not result in liberation of formaldehyde, there was a significant increase in endogenous formaldehyde liberation by microsomes from rats treated at 66 mg/kg-day for 7 weeks, and significantly decreased activities of formaldehyde and propionaldehyde dehydrogenases at 36 and 66 mg/kg-day. In the kidneys, glutathione content and deethylase activity increased and cytosolic formaldehyde dehydrogenase activity decreased. This study found biochemical changes in the liver and kidney, and an increase in relative liver weight, occurring at doses of 7 mg/kg-day or above. However, these changes are considered to be an adaptive response to dimethylformamide, not evidence of a toxic effect.

*Toxicokinetics*

Dimethylformamide is readily absorbed by all routes of exposure. Following an application of 2 ml of liquid dimethylformamide over an area of 100 $cm^2$ on the forearm of volunteers, percutaneous absorption occurred at a rate of 9 $mg/cm^2$/hour (IARC, 1999). A significant amount of percutaneous absorption occurred in volunteers exposed to atmospheres containing 50 $mg/m^3$ of dimethylformamide vapor for 4 hours while breathing fresh air through masks. Under conditions in which volunteers also inhaled the atmosphere, percutaneous absorption accounted for 13-36% of a specific urinary metabolite, *N*-hydroxymethyl-*N* –methyl-formamide (HMMF) (IARC, 1999). Inhalation absorption in human volunteers exposed to concentrations between 10 and 60 $mg/m^3$ for 8 hours or 30 $mg/m^3$ for 8 hours/day on 5 consecutive days, was approximately 90% (IARC, 1999). Analyses of urine recovered 0.3% as unmodified dimethylformamide, 22% as HMMF, 13% as *N*-hydroxymethylformamide and 13% as the mercapturic acid conjugate *N*-acetyl-*S*-*(N*-methylcarbamoyl)cysteine (IARC, 1999).

The primary metabolic pathway for dimethylformamide in humans and rodents is the α-hydroxylation of one alkyl moiety, specifically by cytochrome P450 2E1 to form HMMF (IARC, 1999; Amato et al., 2001). HMMF decomposes to *N*-methylformamide, which undergoes P450-mediated oxidation to form N-acetyl-S-(N-methylcarbamoyl)-cysteine (AMCC) via the reactive carbamoylating intermediate, methylisocyanate (MIC). Humans and animals differ in the proportion of specific urinary metabolites. In 10 volunteers exposed to 60 mg/m$^3$ for 8 hours, 9.7-22.8% of the dose was excreted as AMCC in urine over 72 hours, whereas in rats, mice or hamsters exposed by intraperitoneal injection, only 1.1-5.2% was excreted as AMCC (Mráz et al., 1989). The higher production of AMCC in humans is thought to reflect the higher potential for reactive intermediate generation in the human liver. This metabolic difference appears to account for the greater hepatic sensitivity to dimethylformamide in humans as compared to laboratory animals.

Dimethylformamide and its metabolites readily passed through the placenta and were found in embryonic/fetal tissues following gavage administration of 100 mg/kg of the chemical to 16-hour fasted pregnant Sprague-Dawley rats on GD 12 or 18 (Saillenfait et al., 1997). Fasting, however, is known to affect the pharmacokinetics (and toxicity) of chemicals. No qualitative or quantitative differences were seen between maternal plasma and placenta, amniotic fluid or embryo/fetus in the concentrations of parent compound and metabolites. HMMF was the most prominent metabolite, followed by NMF. In rats dosed on GD 12 and monitored for 8 hours, peak concentrations of parent compound had been attained by the first sampling time, 1 hour, remained fairly constant through the second sampling time, 4 hours, and decreased by the third sampling time, 8 hours. Levels of metabolites increased through the 8 hour sampling period such that HMMF concentrations were equal to or higher than levels of parent compound by 8 hours. In rats dosed on GD 18 and monitored for 24 hours, results were similar, but the major decline in concentrations of parent compound occurred between 8 and 16 hours. In the same study, concentrations of dimethylformamide, HMMF and NMF in the milk of lactating rats were in equilibrium with concentrations in maternal plasma following gavage dosing of the non-fasted dams on day 14 postpartum with 100 mg/kg of dimethylformamide.

A decrease in hepatic cytochrome P450 content has been reported in animals treated with dimethylformamide following bioactivation of dimethylformamide. In an *in vitro* experiment, incubation of 0-20 mM dimethylformamide with hepatic microsomes from pyridine-induced rats resulted in concentration-dependent reductions of cytochrome P450 as high as 28% (Tolando et al., 2001). Electron spin resonance analysis indicated that the suicidal inactivation of P450 was the result of the modification of the prosthetic heme moiety by free radical intermediates of dimethylformamide.

Hemoglobin adducts have been detected in the blood of workers in the polyacrylic fiber industry who were exposed to dimethylformamide (Angerer et al., 1998). Blood levels of hemoglobin with N-methylacarbomoylated valine residues were 100 times higher in occupationally exposed individuals than in the general population. The formation of hemoglobin adducts would be expected to result in deformation of erythrocytes, leading to their removal by the spleen. This process may contribute to the reduced erythrocyte counts observed in the subchronic dietary studies in rats (Kennedy and Sherman, 1986).

*Genotoxicity*

Extensive genotoxicity testing on dimethylformamide yielded generally negative results in bacterial and other non-mammalian systems. Dimethylformamide did not induce reverse mutations in *Salmonella typhimurium* strains TA100 (16 studies), TA1535 (11 studies), TA1537 (12 studies), TA92, TA1530, TA1531, TA1532 and TA1964 with or without metabolic activation, or in strains TA98 (14 studies) or TA1538 (7 studies) without activation (U.S. EPA, 1986; IARC, 1989, 1999). False positive results with activation were reported in a single study on TA98 and TA1538, but all other tests with activation on these strains (14 and 8 studies, respectively) yielded negative results (U.S. EPA, 1986; IARC, 1999). Dimethylformamide did not induce forward or reverse mutations in *Escherichia coli* K-122/343/113 without activation, or reverse mutations in *E. coli* WP2 *uvrA* strains with or without activation (IARC, 1999). With or without activation, dimethylformamide gave negative results in the SOS repair test in *S. typhimurium* TA1535/pSK1002 and in assays for differential toxicity in *Bacillus subtilis* (rec strain) and *E. coli* (DNA-repair deficient strains) (IARC, 1999). Dimethylformamide did not induce forward mutations in *Saccharomyces cerevisiae* XV185-14C or in *Saccharomyces pombe* with or without activation (IARC, 1999). It gave positive results with or without activation in one test for homozygosis by mitotic recombination or gene conversion in *S. cerevisiae* D7, but negative results in 'race XII' and strains D4 and JD1 (IARC, 1999). In tests for differential toxicity in DNA-repair-deficient strains, dimethylformamide gave positive results in *S. cerevisiae* rad and negative results in 'race XII' (IARC, 1999). Dimethylformamide did not induce heritable sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered either in feed or by injection (IARC, 1999).

*In vitro* genotoxicity studies for dimethyformamide in mammalian cells were primarily negative. With or without S9, dimethylformamide did not increase the mutation frequency in human fibroblasts and in three studies on murine L5178Y $tk^{+/-}$ lymphoma cells, but increased the mutation frequency 2-fold in a single study on the lymphoma cells without S9 (IARC, 1999). Without activation, dimethylformamide gave weakly positive results for unscheduled DNA synthesis in cultured primary hepatocytes from Fischer 344 rat, but negative results in other tests on hepatocytes from rat, mouse and hamster (IARC, 1999). Dimethylformamide did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells, rat liver $RL_1$ cells, or, in one study at a concentration of 80 mg/mL, in human lymphocytes; another study reported positive results for chromosomal aberrations in human lymphocytes at a concentration of 7 ng/mL (IARC, 1999). Dimethylformamide did not induce sister chromatid exchanges (SCE) in human lymphocytes without activation or CHO cells with or without activation (IARC, 1999).

*In vivo* genotoxicity studies in mammals provided only weak evidence for genotoxicity. Studies on occupationally-exposed workers yielded mixed results (IARC, 1999). Berger et al. (1985) reported an increased frequency of chromosomal gaps and breaks in 20 workers exposed to mono-, di- and trimethylamines in addition to dimethylformamide (12 $mg/m^3$) compared to 18 unexposed controls (1.4 vs. 0.4%, respectively); however, the authors noted that the control value was low compared to other studies and the study did not address the effect of smoking (IARC, 1999). In a study that obtained blood samples at 4- or 6-month intervals from 40 workers exposed to dimethylformamide and trace amounts of other organic compounds (2-butanone, butyl

acetate, toluene, cyclohexanone and xylene), the frequencies of chromosomal aberrations in peripheral lymphocytes were 3.82, 2.74, 1.59, 1.58 and 1.49%, respectively, following average dimethylformamide exposures of 180, 150, 50, 40 and 30 mg/m$^3$ (Koudela and Spazier, 1981); aberration frequencies in two unexposed control groups were 1.61 and 1.10%. An abstract reported that there was no evidence for an increased chromosomal aberration frequency in occupationally-exposed workers, but no details were provided (IARC, 1999). Seiji et al. (1992) compared the rates of sister chromatid exchanges in peripheral lymphocytes in 22 women occupationally-exposed to dimethylformamide and 22 matched controls. The exposed group was subdivided by level of exposure: 17.4 mg/m$^3$ (high group), 2.1 mg/m$^3$ in combination with an equivalent amount of toluene (medium group) and 0.9 mg/m$^3$ (low group). Compared to the matched controls, the frequency of SCE was significantly higher in the medium and high exposure groups but not in the low exposure group. In mice intraperitoneally injected with dimethylformamide without exogenous activation, micronuclei were induced in the bone marrow of the Kunming strain injected with a dose of 1 mg/kg, but not other strains injected at higher doses: CD (1500 mg/kg), ICR (1600 mg/kg), B6C3F$_1$ (2.5 mg/kg) or BALB/c (2000 mg/kg) (IARC, 1989, 1999). Following intraperitoneal injection of dams with 3 mL/kg of dimethylformamide, there was no evidence for morphological transformation in cultured Syrian hamster embryos (IARC, 1999). An abstract reported no dominant-lethal effect in Sprague-Dawley rats exposed to dimethylformamide by inhalation at a concentration of 900 mg/m$^3$ for 6 hours/day on 5 consecutive days (IARC, 1999). Dimethylformamide gave negative results in a bone marrow cell cytogenetics assay in rats exposed at 1200 mg/m$^3$ for 7 hours or 7 hours/day for 5 days and in a mouse sperm morphology assay (IARC, 1999).

## DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR DIMETHYLFORMAMIDE

No information was located regarding the oral toxicity of dimethylformamide in humans. Oral systemic toxicity data in animals are available from subacute and subchronic studies in rats, mice and gerbils, as summarized in Table 1; chronic oral testing has not been conducted. These studies collectively indicate that the liver is the most sensitive systemic target of oral dimethylformamide, as shown by effects that increased in severity with dose level. The target organ effects included non-adverse changes in rats at 7-235 mg/kg-day (induction of microsomal enzymes and increased liver weight, with no accompanying increases in serum ALT, AST or AP or histological changes) (Becci et al., 1983; Elovaara et al., 1983; Kennedy and Sherman, 1986; Haskell Labs, 1960); slight histological alterations (hepatocellular hypertrophy, increased mitosis) and/or increases in serum ALT in mice at 246-326 mg/kg-day and rats at 422-426 mg/kg-day (Becci et al., 1983; Kennedy and Sherman, 1986; Haskell Labs, 1960), liver necrosis with death in gerbils at >500 mg/kg-day (Llewellyn et al., 1974) and severe hepatic necrosis and fibrosis in rats at 708 mg/kg once weekly (Itoh et al., 1987). Additional considerations in the weight of evidence for liver toxicity are that some subchronic and chronic inhalation studies in rats and mice report that liver enlargement, hepatocellular hypertrophy, and single-cell or focal necrosis of hepatocytes co-occurred at the lower exposures, and more severe hepatic necrosis and other more clearly adverse changes occurred at higher exposures (Malley et al. 1994; Senoh et al. 2003). In addition, occupational studies have reported elevated indices of liver toxicity and suggestive symptoms of

**Table 1. Summary of Non-Cancer Oral Data for Dimethylformamide in Animals**

| Study type<br>Reference | Description | NOAEL<br>(mg/kg-day) | LOAEL<br>(mg/kg-day) | Effects |
|---|---|---|---|---|
| **Subacute exposure**<br>Itoh et al., 1987 | Rats, F344, 8/sex/group<br>Gavage: undiluted, once weekly, 12 weeks<br>0, 708, 945 mg/kg-week | -- | 708 (once weekly)<br>(FEL) | Hepatic fibrosis and severe hepatic necrosis in both sexes, decreased BW gain and depressed activity in males. |
| **Subacute exposure**<br>Kennedy and Sherman, 1986 | Rats, CD, 6 M/group<br>Gavage: 10% in water, nine doses/2 weeks<br>0, 450 mg/kg-day | -- | 450 | "Discomfort" after dosing; variation in size and staining of liver nuclei, frequent mitotic figures, some hepatic cells contained two nuclei (these changes seen in three treated rats examined after the 9$^{th}$ dose but not in three rats following 11-day recovery). |
| **Subchronic exposure**<br>Kennedy and Sherman, 1986; Haskell Labs, 1960 | Rats, CD, 6/sex/group<br>Dietary: 0, 200, 1000, 5000 ppm; 93-94 days<br>M: 0, 16, 77, 426 mg/kg-day<br>F: 0, 17, 85, 422 mg/kg-day | 1000 ppm diet<br>77 (M)<br>85 (F) | 5000 ppm diet<br>426 (M)<br>422 (F) | Increased absolute and relative liver weight and increased hepatocellular mitosis and enlargement. |
| **Subchronic exposure**<br>Becci et al., 1983 | Rats, Wistar, 25/sex/group<br>Dietary: 0, 215, 750, 2500 ppm; 104 days<br>M: 0, 18, 61, 210 mg/kg-day<br>F: 0, 20, 69, 235 mg/kg-day | 2500 ppm diet<br>210 (M)<br>235 (F) | -- | Changes in body and organ weights due to decreased food consumption, not considered adverse; no histological changes. |
| **Subchronic exposure**<br>Becci et al., 1983 | Mice, CD-1, 30/sex/group<br>Dietary: 0, 160, 540, 1850 ppm; 119 days<br>M: 0, 22, 70, 246 mg/kg-day<br>F: 0, 28, 96, 326 mg/kg-day | 540 ppm diet<br>70 (M)<br>96 (F) | 1850 ppm diet<br>246 (M)<br>326 (F) | 66% increase in serum ALT in females, increased absolute and relative liver weight, hepatocytomegaly (histopathologic examinations conducted on all high dose and control mice but only on 5/sex/dose for mid and low dose). |
| **Subchronic exposure**<br>Llewellyn et al., 1974 | Gerbils, 12 females<br>DW: 10,000 ppm, 200 days<br>>500 mg/kg-day; *no controls* | -- | 10,000 ppm DW<br>>500 (FEL) | Necrotic lesions in livers of four gerbils that died (*uncertain whether livers of survivors were examined*). |

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| Study type<br>Reference | Description | NOAEL<br>(mg/kg-day) | LOAEL<br>(mg/kg-day) | Effects |
|---|---|---|---|---|
| **Continuous breeding/ 2-Generation**<br>Fail et al., 1998; NTP, 1992a | Mice, Swiss CD-1, 40 (control) and 20 (treated groups) breeding pairs at start<br>DW: 0, 1000, 4000, 7000 ppm<br><br>$F_0$ M: 0, 185, 610, 1049 mg/kg-day<br>$F_0$ F: 0, 225, 788, 1427 mg/kg-day<br><br>$F_1$ M: 0, 226, 947, 1814 mg/kg-day<br>$F_1$ F: 0, 292, 1098, 2054 mg/kg-day | $F_0$<br>1000 ppm DW<br>185 (M)<br>225 (F)<br><br>$F_1$ pups<br>1000 ppm DW<br>292<br><br>--<br><br>-- | $F_0$<br>4000 ppm DW<br>610 (M)<br>788 (F)<br><br>$F_1$ pups<br>4000 ppm DW<br>1098<br><br>F1 adults<br>1000 ppm DW<br>226 (M)<br>292 (F)<br><br>F2 pups<br>1000 ppm DW<br>292 | Liver weight effects at ≥1000 ppm. Dose-related effects on body weight primarily at mid and high dose; centrilobular hepatocellular hypertrophy (in livers with gross lesions) and decreased fertility at mid and high dose.<br><br>Increased external malformations, particularly of the head, and decreased pre- and post-natal survival, decreased pup weight; crossover $F_0$ mating study showed pup effects were mediated through the dams and included abnormal or incomplete ossification of cranium and sternebrae and brain abnormalities.<br><br>Increased absolute and relative liver weights, centrilobular hepatocellular hypertrophy (in livers with gross lesions). At mid and high doses, also liver weight effects and decreased body weights and decreased fertility; malformations involving abnormal or incomplete ossification of cranium and sternebrae.<br><br>Decreased live F2 pup body weight at low dose. At mid and high doses, decreased live pups, increased pup malformations. |
| **Developmental**<br>Hellwig et al., 1991 | Rats, SD, 19-23 pregnant rats/group<br>Gavage: in water<br>166, 503, 1510 mg/kg-day GD 6-15:<br>*Controls were untreated* | Maternal<br>--<br><br>Developmental<br>166 | Maternal<br>166<br><br>Developmental<br>503 | Reduced placental weight (4%), dose related through higher doses; dose-related decreases in maternal BW at mid and high doses *but food consumption not reported.*<br><br>Increased resorptions (per litter); decreased fetal BW and increased skeletal anomalies (*but data not analyzed per litter*). |

**Table 1. Summary of Non-Cancer Oral Data for Dimethylformamide in Animals**

| Study type<br>Reference | Description | NOAEL<br>(mg/kg-day) | LOAEL<br>(mg/kg-day) | Effects |
|---|---|---|---|---|
| **Developmental**<br>Hellwig et al., 1991 | Mice, NMRI, 24 pregnant mice/group<br>Gavage: in water<br>182, 548 mg/kg-day GD 6-15:<br>*Controls were untreated* | Maternal<br>548<br><br>Developmental<br>182 | <br><br>Developmental<br>548 | No obvious effect on maternal BW and BW gain *but statistical analysis not performed and data not provided*; no clinical signs.<br><br>Reduced fetal length at low dose; fetal BW and length decreased and anomalies increased at high dose; *but data not analyzed per litter.* |
| **Developmental, pre- and post-natal**<br>Fritz and Giese, 1990; Ciba-Geigy, 1979 | Rats, SD, 10/group/duration<br>Gavage: in water<br>0, 750 mg/kg-day GD 10-14 | Maternal<br>No data<br><br>Developmental<br>-- | Maternal<br>No data<br><br>Developmental<br>750 | *Maternal endpoints including BW and food consumption not reported.*<br><br>Reduced fetal BW, delayed ossification lasting through PND 5. |
| **Developmental**<br>Merkle and Zeller, 1980 | Rabbits, Russian, 11-18/group<br>Gavage (in water)<br>0, 43.8, 64.3, 188.9 mg/kg-day GD 6-18 | Maternal and Developmental<br>64.3 | Maternal and Developmental<br>188.9 | Decreased maternal food consumption and weight gain; decreased fetal BW, increased percentage of fetuses and litters with malformations, *but litter incidences of individual malformations not reported.* |
| **Developmental**<br>Saillenfait et al., 1997 | Rats, SD, 22-24 pregnant rats/group<br>Gavage: 0, 2.5, 5, 10, 15 % in water<br>0, 50, 100, 200, 300 mg/kg-day GD 6-20 | Maternal and Developmental<br>50 | Maternal and Developmental<br>100 | Decreased maternal food consumption and BW gain; decreased fetal BW.<br><br>At higher doses, dose-related depression of maternal food consumption and maternal and fetal BW gain and increased fetal skeletal variations related to delayed ossification. |

BW = body weight, DW = drinking water, F = female, M = male

liver effects in exposed workers. The chronic RfC on IRIS is based on a LOAEL for "digestive disturbances and minimal hepatic changes suggestive of liver abnormalities" in occupational studies (U.S. EPA, 2007).

Critical effect levels for hepatic effects were identified in the Becci et al. (1983) and Kennedy and Sherman (1986; Haskell Labs, 1960) subchronic dietary studies in rats and mice. The Becci et al. (1983) study used larger numbers of animals (25-30/sex/dose), was comprehensive in scope and identified hepatic NOAELs of 210-235 mg/kg-day in rats and 70-96 mg/kg-day in mice (for increased liver weight in the absence of serum enzyme and histological changes), no LOAELs in rats, and LOAELs of 246-326 mg/kg-day in mice (for hepatocytomegaly and increased serum ALT). The Kennedy and Sherman (1986; Haskell Labs, 1960) study evaluated a small number of rats (6/sex/dose) and endpoints and identified NOAELs of 77-85 mg/kg-day (for increased liver weight in the absence of serum enzyme and histological changes, and LOAELs of 422-426 mg/kg-day (for increased hepatocellular mitosis and enlargement). The histology and serum enzyme data indicate that mice are more sensitive than rats, as shown by the LOAELs of 246 mg/kg-day (males)-326 mg/kg-day (females) in mice and 422-426 mg/kg day in rats. The highest systemic NOAELs in mice are 70 mg/kg-day (males) and 96 mg/kg-day (females) (Becci et al., 1983).

A continuous breeding/2-generation study of dimethylformamide administered in the drinking water to mice (Fail et al., 1998; NTP, 1992a) found systemic and fetal effects at dose levels similar to the 246-326 mg/kg-day LOAEL for liver toxicity in the Becci et al. (1983) subchronic dietary study in mice, as summarized in Table 1. In the $F_1$ mice, absolute and relative liver weights were increased, and hepatocellular hypertrophy was observed (in livers with gross lesions) at the lowest dose (226 and 292 mg/kg-day in males and females, respectively); $F_2$ pup weights were also decreased at this dose level. A crossover mating study with the $F_0$ mice had determined that effects on the pups were mediated through the dams. A NOAEL for systemic toxicity was not identified, and the developmental effects occurred at minimally maternally toxic doses.

Developmental toxicity studies, all of which were conducted by gavage, have identified LOAELs for maternal and developmental toxicity of dimethylformamide that are lower than those seen in the subchronic and reproductive studies conducted using dietary or drinking water exposure (Table 1). Gestational exposure of Sprague-Dawley rats by gavage resulted in maternal toxicity (reduced food consumption and body weight gain) and developmental toxicity (reduced fetal body weight) at $\geq$100 mg/kg-day and increases in skeletal variations indicative of delayed ossification at $\geq$200 mg/mg-day (Saillenfait et al., 1997); the NOAEL for maternal and developmental toxicity was 50 mg/kg-day. In a developmental gavage study in Sprague-Dawley rats, 166 mg/kg-day was a minimal maternal reproductive LOAEL for reduced placental weight (Hellwig et al., 1991), which was a likely precursor to reduced fetal weight gains at higher doses. In rabbits, 189 mg/kg-day was teratogenic (increased incidence of total malformations), fetotoxic (decreased fetal weight) and maternotoxic (reduced body weight gain and food intake), while 64 mg/kg-day was a NOAEL for developmental and maternal effects (Merkle and Zeller, 1980). The lowest LOAEL is 100 mg/kg-day in rats and the corresponding NOAEL is 50 mg/kg-day (Saillenfait et al., 1997).

Effects on fetal weight and ossification in the gavage studies, however, may have been secondary to maternal effects. Data for maternal food consumption and maternal and fetal body weight in the study of Saillenfait et al. (1997) are presented in Table 2. The effect on corrected maternal body weight gain (corrected by subtracting the weight of the gravid uterus) as compared with the effect on fetal body weight indicates that the effect was primarily on the dams. Effects on fetal ossification occurred at dose levels ≥200 mg/kg-day, where the corrected maternal body weight gain was decreased by 79-90% and the fetal body weight was decreased by 12-14%, and are consistent with delayed ossification, which is likely related to the depression of maternal and fetal body weight (e.g., Fleeman et al., 2005; Hood, 2005).

**Table 2. Food Consumption, Body Weight, and Ossification Effects in Sprague-Dawley Rats Treated by Gavage with Dimethylformamide on Days 6 to 20 of Gestation (Saillenfait et al., 1997)**

| Dose, mg/kg-day | 0 | 50 | 100 | 200 | 300 |
|---|---|---|---|---|---|
| Maternal food consumption, g/dam/day (% decrease) | 28.7±0.7[a] | 28.6±0.6 (0.3)[b] | 24.4±0.6[c] (15.0) | 21.8±0.6[c] (24.0) | 22.7±0.6[c] (20.9) |
| Maternal body weight gain, g (% decrease) | | | | | |
| GD 6-21 | 154±7 | 148±8 (3.9) | 128±5[c] (16.9) | 102±5[c] (33.8) | 92±5[c] 40.3) |
| GD 6-21 minus gravid uterus | 43±5 | 48±3 | 25±4[c] (41.9) | 9±4[c] (79.1) | 4±4[c] (90.7) |
| Fetal Body weight/litter, g (% decrease) | 5.54±0.05 | 5.52±0.04 (0.3) | 5.30±0.05[c] (4.3) | 4.87±0.05[c] (12.1) | 4.76±0.06[c] (14.1) |
| Supraoccipital: absent or incomplete ossification Litter incidence | 0 | 2/20 | 7/19 | 16/19[c] | 20/20[c] |
| Sternebrae: absent or incomplete ossification, Litter incidence | 2/16 | 7/20 | 7/19 | 11/19[d] | 13/20[c] |

[a]Mean ± standard error of the mean
[b]Percent decrease relative to vehicle control value
[c]Significantly different from vehicle control value, $p<0.05$
[d]Significantly different from vehicle control value, $p<0.01$

Consideration was given to whether the results seen in the gavage developmental studies indicate an increased sensitivity of pregnant animals to dimethylformamide, or whether they indicate that once a day bolus dosing is more toxic than routes of exposure more relevant to human exposure (i.e., dietary and drinking water exposures). The results from the continuous breeding/2-generation drinking water study in pregnant mice (Fail et al., 1998; NTP 1992a) were compared to those from the Becci et al. (1983) subchronic dietary toxicity study in nonpregnant

mice. This comparison suggests that systemic toxicity (liver effects) in females occurred at approximately the same exposure level in the reproductive study (292 mg/kg-day, resulting from 1000 ppm in the drinking water) as in the subchronic study (326 mg/kg-day, resulting from 1850 ppm in the diet). Thus, pregnant mice do not seem more sensitive to dimethylformamide than nonpregnant female mice.

Three studies were considered as the basis for the subchronic RfD – the developmental toxicity study by Saillenfait et al. (1997), the continuous breeding/2-generation study by Fail et al. (1998; NTP, 1992a), and the subchronic toxicity study by Becci et al. (1983).

As noted above (see also Table 1), gavage administration of dimethylformamide in studies by Saillenfait et al. (1997) and others has been shown to cause developmental toxicity in rats, mice and rabbits, generally at doses associated with maternal toxicity. The presence of maternal toxicity complicates the interpretation of the developmental toxicity findings. As noted in U.S. EPA's Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), "when adverse developmental effects are produced only at doses that cause minimal maternal toxicity…, the developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. At doses causing excessive maternal toxicity…, information on developmental effects may be difficult to interpret and of limited value." These guidelines also note that where maternal toxicity is seen at dose levels lower than those producing developmental toxicity or at exposure levels lower than that in other evaluations of adult toxicity, "this implies that the pregnant female is likely to be more sensitive than the nonpregnant animal," and that data from such studies should be used in the overall assessment of risk.

The possible influence of gavage administration merits some consideration. The analysis of data for pregnant mice from Fail et al. (1998; NTP 1992a) that involved nongavage (drinking water) administration with mouse data from Becci et al. (1983) that also involved nongavage (dietary) administration suggested that pregnant mice were not more sensitive to dimethylformamide than nonpregnant animals. Therefore, it is possible that the lower LOAEL in the developmental gavage toxicity studies may been due to enhancement of dimethylformamide toxicity by bolus dosing. However, overt fetal malformations were observed in multiple developmental toxicity studies following dimethylformamide exposure, lending uncertainty as to whether maternal toxicity was a major factor in the observed toxicity in some studies, thus data from developmental (gavage administration) studies was considered in the derivation of a subchronic p-RfD.

Of the available developmental toxicity studies, Saillenfait et al. (1997) reported maternal and developmental toxicity at the lowest doses. In this study, pregnant SD rats received dimethylformamide by gavage in drinking water at doses ranging from 50 to 300 mg/kg-day on GD 6-20. Investigators reported dose-related reductions in maternal body weight gain and food consumption in dams, reductions in fetal body weight, and increased incidences of two skeletal variations (absent or incompletely ossified supraoccipital and sternebrae) at 100 mg/kg-day (LOAEL); the NOAEL was 50 mg/kg-day. Although effects on the fetus may have been secondary to effects on maternal body weight gain, the study nevertheless provides clear evidence

of treatment-related toxicity at a dose of 100 mg/kg-day. These findings are supported by Merkle and Zeller (1980), which identified a NOAEL and LOAEL of 64.3 and 189 mg/kg-day, respectively, for maternal and developmental toxicity in the rabbit.

A provisional subchronic RfD of 0.5 mg/kg-day for dimethylformamide based on the Saillenfait et al. (1997) study was derived by applying an uncertainty factor of 100 (10 to extrapolate from rats to humans and 10 to protect sensitive individuals) to the rat NOAEL of 50 mg/kg-day. An additional uncertainty factor for database limitations was not applied, since reproductive and developmental effects have been adequately studied. The derivation is as follows:

subchronic p-RfD = NOAEL / UF
= 50 mg/kg-day / 100
= 0.5 mg/kg-day

The continuous breeding/2-generation study identified a LOAEL for developmental toxicity (decreased $F_2$ pup weight) at the same dose level as liver effects in the dams (increased liver weight and hepatocellular hypertrophy), and did not identify a NOAEL for either developmental or systemic toxicity (Fail et al., 1998; NTP, 1992a). The investigators concluded that maternal toxicity contributed to fetal and neonatal problems; however, reproductive effects and more severe developmental effects, including skeletal and visceral malformations, that occurred at the two higher exposure levels in this study did not appear to reflect simple developmental delays. Thus, the LOAEL of 292 mg/kg-day for developmental toxicity (decreased $F_2$ pup weight) from the continuous breeding/two generation study of Fail et al. (1998; NTP, 1992a) was considered for subchronic p-RfD derivation.

A provisional subchronic RfD of 0.3 mg/kg-day for dimethylformamide based on the Fail et al. (1998; NTP, 1992a) study was derived by applying an uncertainty factor of 1000 (10 to extrapolate from a LOAEL to a NOAEL, 10 to extrapolate from mice to humans and 10 to protect sensitive individuals) to the mouse LOAEL of 292 mg/kg-day for reduced $F_2$ pup weight. As noted above, an additional uncertainty factor for database limitations was not considered necessary because reproductive and developmental effects have been adequately studied. The derivation is as follows:

subchronic p-RfD = LOAEL / UF
= 292 mg/kg-day / 1000
= 0.3 mg/kg-day

Finally, consideration was given to liver toxicity as the basis for the subchronic p-RfD. The most appropriate study was considered the Becci et al. (1983) study, which was a robust study of systemic toxicity that identified liver toxicity at relatively low doses. The highest NOAEL (96 mg/kg-day) for hepatotoxicity in female mice from Becci et al. (1983) was used to derive a subchronic p-RfD. The NOAEL for the female mouse was the higher of the NOAELs for the male (70 mg/kg-day) and female (96 mg/kg-day) mouse in this study.

A provisional subchronic RfD of 1 mg/kg-day for dimethylformamide based on the Becci et al. (1983) study was derived by applying an uncertainty factor of 100 (10 to extrapolate from rats to humans and 10 to protect sensitive individuals) to the rat NOAEL of 96 mg/kg-day. As noted above, an additional uncertainty factor for database limitations was not considered necessary. The derivation is as follows:

subchronic p-RfD = NOAEL / UF
= 96 mg/kg-day / 100
= 0.96 mg/kg-day or 1 mg/kg-day

Candidate subchronic p-RfDs based on study data from Saillenfait et al. (1997), Fail et al. (1998; NTP, 1992a), and Becci et al. (1983) yielded values ranging from 0.3 to 1 mg/kg-day – a range of only 3.3-fold. The **subchronic p-RfD of 0.3 mg/kg-day** based on developmental effects as reported by Fail et al. (1998; NTP, 1992a), which represents the most sensitive effect, is selected as the subchronic p-RfD.

Confidence in the critical study is medium. The study examined a suitable number of endpoints and was adequately documented. Confidence in the database is high. Supporting data are available from subchronic studies, as well as developmental studies in multiple species. The oral database is also supported by numerous studies conducted for the inhalation route. Medium confidence in the subchronic p-RfD for dimethylformamide results.

No chronic oral systemic toxicity studies are available for derivation of the chronic p-RfD. The same three studies considered for derivation of the subchronic p-RfD were therefore considered as the basis for chronic p-RfD derivation. The p-RfD of 0.3 mg/kg-day based on reduced pup weight in the mouse in the continuous breeding/2-generation study (Fail et al., 1998; NTP, 1992a) and the p-RfD of 0.5 mg/kg-day based on developmental effects in the rat (Saillenfait et al., 1997) are considered appropriate for the assessment of chronic exposure without addition of an uncertainty factor for extrapolation to chronic exposure. The developmental period is recognized as a susceptible lifestage where exposures during certain time windows are more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991b).

A provisional chronic RfD of 0.1 mg/kg-day for dimethylformamide based on the Becci et al. (1983) study is derived by applying an uncertainty factor of 1000 (10 to extrapolate from mice to humans, 10 to protect sensitive individuals and 10 to extrapolate from subchronic to chronic duration) to the NOAEL as follows:

Chronic p-RfD = NOAEL / UF
= 96 mg/kg-day / 1000
= 0.096 or 0.1 mg/kg-day

Candidate chronic p-RfDs based on study data from Fail et al. (1998; NTP, 1992a), Saillenfait et al. (1997), and Becci et al. (1983) yielded values ranging from 0.1 to 0.5 mg/kg-day – a range of only five-fold. The **chronic p-RfD of 0.1 mg/kg-day** based on liver toxicity as

reported by Becci et al. (1983), which represents the most sensitive effect, is selected as the chronic p-RfD.

Confidence in the critical study is medium, as it was well-conducted and reported, and examined a suitable number of endpoints. Confidence in the database is medium because although it includes oral subchronic, reproductive and developmental studies in multiple species, and is supported by an extensive inhalation database, it does not include a chronic oral study. Medium confidence in the chronic p-RfD for dimethylformamide results.

## DERIVATION OF PROVISIONAL SUBCHRONIC INHALATION RfC VALUE FOR DIMETHYLFORMAMIDE

A **chronic RfC of 0.03 or 3E-2 mg/m$^3$** is available for dimethylformamide on IRIS (U.S. EPA, 2007). The RfC, which was verified 8/23/90, was derived based on a LOAEL of 22 mg/m$^3$ (duration adjusted to 7.9 mg/m$^3$) for digestive disturbances and minimal hepatic changes suggestive of liver abnormalities in workers exposed for an average of 5 years (Cirla et al., 1984).

Epidemiological studies demonstrate that dimethylformamide exposure is associated with hepatic toxicity in humans (Cirla et al., 1984; Fiorito et al., 1997; Cai et al., 1992; Wrbitzky, 1999; Lauwerys et al., 1980; Yonemoto and Suzuki, 1980; Redlich et al., 1987a, b, 1988; Riely et al., 1988; Reinl and Urban, 1965; Tolot et al., 1968; Chary, 1974). Several of these studies found hepatotoxicity indicated by effects on serum biomarkers, such as GGT, ALT, AST, AP and bilirubin (Cirla et al., 1984; Fiorito et al., 1997; Wrbitzky, 1999; Redlich et al., 1987a, b, 1988; Riely et al., 1988), and liver biopsy (Redlich et al., 1987a, b, 1988; Riely et al., 1988; Tolot et al., 1968). Although other studies found no effect on serum biomarkers (Catenacci et al., 1984; Cai et al., 1992; Lauwerys et al., 1980; Yonemoto and Suzuki, 1980; Massmann, 1956a; Di Lorenzo and Graziolo, 1972), many of these did find subjective evidence of liver toxicity (e.g., digestive impairment, alcohol intolerance) (Cai et al., 1992; Lauwerys et al., 1980; Yonemoto and Suzuki, 1980). Subjective symptoms indicative of hepatotoxicity were also reported in the studies that found serum biomarker changes (Cirla et al., 1984; Fiorito et al., 1997; Wrbitzky, 1999; Redlich et al., 1987a, b, 1988; Riely et al., 1988) and in other studies (Chary, 1974). The reports indicate that dimethylformamide concentrations as low as 6-20 mg/m$^3$ may produce these effects. Cirla et al. (1984) found significantly increased prevalence of abnormally high GGT levels (and also non-significant increases for abnormally elevated ALT, AST and enlarged liver) and increased subjective complaints potentially related to hepatotoxicity in 100 workers exposed for an average of 5 years to a mean concentration of 22 mg/m$^3$, compared with a pair-matched control group. Fiorito et al. (1997) found significantly increased prevalence for abnormally elevated ALT, AST and GGT, as well as significantly increased mean values for these biomarkers, and increased subjective complaints indicative of hepatotoxicity in a group of 75 workers exposed to an average of 20 mg/m$^3$ for up to 3.8 years. Increased subjective indicators of liver dysfunction, but no effect on serum biochemistry, was reported for 207 workers in a polyurethane plant who were exposed to levels up to 21 mg/m$^3$ for several years, compared with 143 non-exposed workers (67 men and 76 women) in the same plant (Cai et al., 1992). Analysis by department (exposure level) found that the prevalence of self-reported reduced alcohol tolerance was significantly elevated in

subjects exposed to levels of 2 ppm (6 mg/m$^3$) or above, but not average exposures of 0.1-0.6 ppm (0.3-1.8 mg/m$^3$). This study identified a LOAEL of 6 mg/m$^3$ and a NOAEL of 1.8 mg/m$^3$ based on subjective symptoms of hepatic effects in workers exposed to dimethylformamide.

The inhalation toxicity database for animals supports the identification of the liver as the main target organ of dimethylformamide and indicates that humans may be more vulnerable to dimethylformamide than the other tested species. In CD-1 mice exposed for 18 months, 75 mg/m$^3$ was a LOAEL for liver histopathology (centrilobular hepatocellular hypertrophy and necrosis) (Malley et al., 1994). In the companion study in rats exposed for 2 years, 75 mg/m$^3$ was a NOAEL and 300 mg/m$^3$ was a LOAEL for increased relative liver weights and serum enzyme values (Malley et al., 1994). Subchronic toxicity studies on dimethylformamide indicate that rodents may be more susceptible than cynomolgous monkeys to hepatic effects. Thirteen-week bioassays identified hepatic LOAELs of 300-600 mg/m$^3$ in rats (liver cell necrosis) and 150 mg/m$^3$ in mice (increases in centrilobular hepatocellular hypertrophy and liver weight) (NTP, 1992b; Lynch et al., 2003; Senoh et al., 2003). Hepatic effects were also seen at higher concentrations in other subchronic studies in rodents (Craig et al., 1984; Tanaka, 1971; Massmann, 1956b). No adverse effects were observed in cynomolgous monkeys exposed to concentrations as high as 1500 mg/m$^3$ for 13 weeks, although the statistical power of the study was limited due to the small group sizes (3/sex/group) (Hurtt et al., 1992). Gestational inhalation exposure studies in several species reported that developmental effects occurred at maternally toxic concentrations. In rabbits, 150 mg/m$^3$ was a LOAEL for maternal toxicity (reduced body weight gain) and fetal toxicity (minimal increase in developmental anomalies) and the NOAEL for both was 50 mg/m$^3$ (Hellwig et al., 1991). In CD rats, 90 mg/m$^3$ was the maternal and fetal NOAEL and 900 mg/m$^3$ was the LOAEL for reduced body weight gain in dams and reduced body weight in fetuses (Lewis et al., 1992). In Sprague-Dawley rats exposed under nonstandard protocols (for a total of 13 or 16 gestational days), 860 mg/m$^3$ was a LOAEL for reduced body weight gain in dams and reduced fetal weight and increased skeletal variations in fetuses (Hellwig et al., 1991).

The occupational studies of Cirla et al. (1984), Fiorito et al. (1997) and Cai et al. (1992) are chosen as co-principal studies for the subchronic RfC for dimethylformamide. The Cirla et al. (1984) and Fiorito et al. (1997) studies both reported elevated liver enzymes and symptoms indicative of liver abnormalities (alcohol intolerance, gastrointestinal disturbances) at similar 8-hour TWA LOAELs: 22 mg/m$^3$ in Cirla et al. (1984) and 20 mg/m$^3$ in Fiorito et al. (1997). Cai et al. (1992) found subjective evidence of liver toxicity (e.g., digestive impairment, alcohol intolerance), without serum enzyme changes, in workers with an average exposure of 13.7 mg/m$^3$ over several years, and also did a dose-response analysis that identified a LOAEL of 6 mg/m$^3$ and NOAEL of 1.8 mg/m$^3$ for prevalence of self-reported reduced alcohol tolerance. These studies together define a LOAEL of 6-22 mg/m$^3$ and a NOAEL of 1.8 mg/m$^3$ for hepatotoxicity in workers subchronically exposed to dimethylformamide. Because of self-reported reduced alcohol intolerance, the dose-response for hepatotoxicity is questionable. The LOAEL (20 mg/m$^3$) identified in Fiorito et al. (1979) for elevated liver enzyme changes represents critical liver function, thus considered as the POD for derivation of the subchronic p-RfC. The subchronic LOAEL is adjusted for duration by multiplying by the ratio of the reference breathing volume for

light activity during occupational exposure to the reference daily breathing volume (10 $m^3$/ 20 $m^3$) and by the ratio of workdays to weekdays (5/7) (U.S. EPA, 1994b), as follows:

$$\begin{aligned} LOAEL_{ADJ} &= (LOAEL)(VOL_{OCCUP}/VOL_{DAILY})(DAYS_{EXPOSED}/DAYS_{WEEK}) \\ &= (20\ mg/m^3)\ (10\ m^3/20\ m^3)\ (5/7) \\ &= 7.14\ mg/m^3 \end{aligned}$$

The provisional **subchronic RfC of 7E-2 mg/$m^3$** for dimethylformamide is derived by applying an uncertainty factor of 100 (10 for human variability to the duration-adjusted subchronic LOAEL and 10 for use of a LOAEL). An additional uncertainty factor for database limitations was not applied, since the database included adequate human studies to define a NOAEL and LOAEL, and supporting animal data for systemic and developmental effects that showed humans to be more sensitive to liver effects than effects noted in animals. The derivation is as follows:

$$\begin{aligned} \text{subchronic p-RfC} &= LOAEL_{ADJ} / (UF) \\ &= (7.14\ mg/m^3) / (100) \\ &= \mathbf{0.07\ or\ 7E\text{-}2\ mg/m^3} \end{aligned}$$

Confidence in the key subchronic studies is medium-to-high because they carefully evaluated specific endpoints relevant to the primary target organ in an adequate number of exposed workers and controls, were well-designed, well-conducted and adequately-documented, but exposure concentrations and durations were not thoroughly characterized. Confidence in the database is medium-to-high. Supporting human and chronic, subchronic and developmental toxicity studies in multiple species are available for inhalation exposure, but reproductive toxicity has been studied only by oral exposure. Medium-to-high confidence in the provisional subchronic RfC for dimethylformamide results.

## PROVISIONAL CARCINOGENICITY ASSESSMENT FOR DIMETHYLFORMAMIDE

### Weight-of-Evidence Descriptor

Although reports of clusters of testicular cancer in workers exposed to dimethylformamide (combined inhalation and dermal exposure) were a matter of concern (Ducatman et al., 1986; Levin et al., 1987), subsequent investigations failed to confirm any association between dimethylformamide exposure and cancer (Calvert et al., 1990; Chen et al., 1988; Walrath et al., 1989; IARC, 1999). Inhalation exposure to dimethylformamide at concentrations as high as 1200 $mg/m^3$ did not induce tumor formation in rats exposed for 2 years or in mice exposed for 18 months (Malley et al., 1994). An extensive array of *in vitro* and *in vivo* genotoxicity assays on dimethylformamide yielded largely negative results (IARC, 1999). On the basis of inadequate evidence in humans and negative evidence in adequate inhalation assays in rats and mice (Malley et al., 1994), and in accordance with U.S. EPA (2005) guidelines, the data are inadequate for an assessment of human carcinogenic potential for dimethylformamide.

**Quantitative Estimates of Carcinogenic Risk**

The absence of positive carcinogenicity data for dimethylformamide precludes the calculation of quantitative estimates of carcinogenic risk.

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