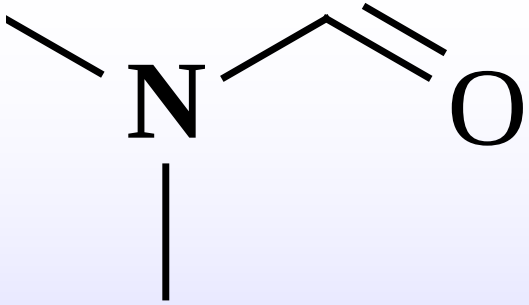
N’N-Dimethylformamide



Synonym: DMF

CAS No: 68-12-2

Physical-Chemical Data

- **Description: colorless to slightly yellow liquid with faint amine odor**
- **Molecular formula: $CHON(CH_3)_2$**
- **Molecular weight: 73.09**
- **Boiling point: 153 C**
- **Solubility: Miscible with water and most common organic solvents**
- **Conversion factor: mg/m^3 = 3.0 ppm**
- **Vapor pressure: 22.5 mmHg at 20 C**
  - Compare to carbon tetrachloride 90.0 mmHg

Occurrence & Use

- **DMF solutions used in a variety of industries**
  - Aircraft maintenance; leather tanning; pesticides and plastics production; adhesive manufacture; synthetic leathers; pharmaceuticals; processing polymer fibers, films and surface coatings
- **US production volume: 100-500 million pounds (2002)**
- **Air emissions in California (2006)**
  - 4.61 tons reported in California Toxics Inventory

Carcinogenicity Studies of DMF

- **Studies in humans**
  - Leather tanners & Navy F4 aircraft repairmen
    - Two cancer cluster investigations
    - One each of case-control and cohort
  - DMF production and use facility employees
    - One each of case-control and cohort

- **Studies in animals**
  - Rats: drinking water studies
  - Mice: two sets of long-term inhalation studies in males and females
  - Rats: two sets of long-term inhalation studies in males and females

Original Cluster: Navy F4 Aircraft Repairmen

- **Cluster investigation of testicular germ cell tumors**
  - Three males among 153 workers at one facility
  - Four more cases found among workers at a second F4 aircraft maintenance facility
  - No cases at third facility that had no DMF exposure
- **Exposed for 4-19 years**
- **Repairmen dripped solvent mixture containing 80% DMF onto cables**
- **No DMF air measurements**
  - Frumin *et al*. (1989) speculated that exposures were >10 ppm

Leather Tanners

- **Cluster investigation of testicular germ cell tumors at leather tannery**
  - 3 cases
- **Exposed for 8-14 years**
- **Worked on spray line**
  - Spread dyes on leather with paddles while leaning close to hide (Levin *et al*., 1987)
- **No DMF air measurements**
  - Frumin *et al*. (1989) speculated that exposures were >10 ppm before being removed from process

Follow-up of Leather Tanners: Case-Control Study (Frumin *et al*., 1989)

- **Case-control study in whole county**
  - Cases from New York State Cancer Registry
    - Diagnosed from 1974-1987
    - 7 additional cases for total of 10
  - Control group of 129 men who developed another type of cancer during same years
  - Five of 10 cases (50%) and 17 of 129 controls (13%) in “leather related occupations” (Frumin *et al*., 1989)
- **Odds Ratio = 5.8 (95% CI 1.5-22.0)**

Follow-up of Leather Tanners: Case-Control Study (NY State DOH)

- **Report same information as Frumin *et al*. (1989)**
- **Slightly larger control group (144 men)**
- **Description and discussion of controls and cases**
  - Many controls missing occupation information (removed from analysis)
    - More prevalent in younger controls
  - Controls older than cases
    - Less likely to have testicular germ cell cancer
    - Potentially overestimates risk of testicular cancer
  - Percent of leather tanners higher in controls
    - Potentially obscures effects from leather tanning occupation exposure
- **Potential bias in unknown direction**

Follow-up of Leather Tanners: Cohort Study (Frumin *et al*., 1989; Calavert *et al*., 1990)

- **Cohort study at leather tannery of 80 workers**
  - Expected number of cases calculated
    - Based on NY State incidence rates
    - Person-years at risk from 1975-1987
- **Standardized Incidence Ratio = 40.5 (95% CI 8.1-118.4)**

DMF Production and Use Facilities Worker Studies

- **Cohort study by Chen *et al*. (1988)**
  - One plant
    - Manufacturing acrylic fibers
- **Case-control study by Walrath *et al*. (1989)**
  - Four plants
    - One DMF production and three manufacturing plants

DMF Production and Use Facilities

	Plant A	Plant B	Plant C	Plant D
Type of facility	DMF production	Used as solvent: acrylic fibers manufacture	Used as solvent: acrylic fibers manufacture	Used as ink solvent: tinting of plastic sheeting
Year of start	1938	1958	1950	1958
Percent exposed to DMF	7.7%	44.7%	83.2%	18.5%
Average exposure level in plant	All were <1 ppm	Split evenly: <1 1 to <2 or 2 to < 10 ppm	> 50% were 2 to < 10 ppm	> 50% were <1 ppm
Average annual employee population (1956-1985)	2052	2246	2276	2150

Cohort Study
(Chen *et al*., 1988)

- **Plant manufactures acrylic fibers**
  - Acrylonitrile (ACN) co-exposures for some employees
  - Only DMF exposure for some workers
- **Cases from Du Pont Cancer Registry**
  - Only cancers diagnosed *while* at Du Pont
- **2530 exposed, 47 cancer cases (1.8%)**
- **1130 unexposed, 17 cancer cases (1.5%)**
- **Exposure classification**
  - Grouped as “ever” vs. “never”
  - Occurred between 1950 and 1970

DMF-Only Cohort Study: Results (Chen *et al*., 1988)

- **Expected counts based on Du Pont cancer incidence rates**
- **One significant association ($p<0.05$) in DMF-only cohort**
  - Buccal cavity & pharynx (wage and total)
- **Other cancers examined with no reported associations**
  - All types combined, lung, malignant melanoma, prostate, stomach, intestine, nervous system, lymphohematopoietic, bladder and all others

DMF-Only Cohort Study: Results (Chen *et al*., 1988)

- **Expected counts using National Cancer Institute's SEER cancer incidence rates**
- **Buccal cavity/pharynx (9 cases)**
  - Significantly higher than expected 3.3 cases
  - Six cases with "high" exposure
    - Contact with DMF containing liquids, air concentration often >10 ppm
  - Three cases with "moderate" exposure
    - Intermittent liquid contact and air concentration >10 ppm more than once a week
- **Malignant melanoma (5 cases)**
  - Significantly higher than expected 1.6 cases
  - All five cases in "high" exposure category
- **Expected counts with SEER rates not significant for other cancers**

Case-Control Study (Walrath *et al*., 1989)

- **Included all four plants, three manufacturing and one production**
- **Cases from Du Pont Cancer Registry**
  - Only cancers diagnosed *while* at Du Pont
- **Co-exposure to ACN not discussed**
- **Controls matched by plant, age, sex & payroll type**
  - Activities varied by plant
  - Plant was used as surrogate of exposure
- **Five cancers examined: Buccal cavity/pharynx, liver, prostate, testis, and skin**
- **Odds ratios reported by plant**

Case-Control Results
(Walrath *et al*., 1989)

- **Small number of cases for each cancer—limited statistical power**
- **Prostate cancer (4 cases) at Plant D was only significant association**
  - Odds Ratio = 8.04 (90% CI = 1.04-62.3)
- **Logistic regression trend for malignant melanoma**
  - By increasing exposure category (none, present, low and moderate)

Biomarkers & Bioaccumulation (Chang *et al*., 2004; 2005)

- **Body burden of DMF studied**
  - Two urinary biomarkers (DMF, NMF *)
  - Personal air and dermal (hands and arms) DMF measurements
  - Occupational exposure in multiple industries with different exposure
  - One-day and one-week exposure studies
- **Higher levels of NMF for the workers with dermal exposure**
- **Dermal DMF exposure results in bioaccumulation**

* NMF = N-methylformamide

Analysis of Chen *et al.* (1988)

- **Chen *et al*. (1988) report using Poisson distribution with 2 tails and p ≤ 0.1 cutoff**
  - Two-tail with p ≤ 0.1 cutoff and one-tail with p ≤ 0.05 are identical
- **Unclear why some associations not significant**
- **Associations not reproducible**
- **SIRs not mentioned in publication or reported in tables**
- **Poisson distribution**
  - Mean of distribution is the expected count
  - Probability of observed count or greater
  - *A priori* testing association of cancer with DMF (won't prevent cancers) so one tail assumption appropriate
- **Chi-squared distribution**
  - For expected counts greater than two
  - Inherently two tailed based on shape of distribution (skewed distribution with only one tail)

OEHHA Analysis Results: Chen *et al.* (1988)

- **Both distributions qualitatively provide similar results**
- **Significant associations in DMF cohort**
  - Poisson distribution: Buccal cavity/pharynx, stomach
    - Malignant melanoma (SEER)
  - Chi-square distribution: Buccal cavity/pharynx, malignant melanoma, prostate, stomach
- **Based on methods described, find additional significant associations**

DMF Only Cohort: Chi-Square

Cancer Type	Wage			Salary			Total		
	Obs	Exp	p-value	Obs	Exp	p-value	Obs	Exp	p-value
All cancers	34	25.3	0.084	13	14.7	0.657	47	40	0. 268
Bucc.pharynx	**8**	**1**	**<0.001****	1	0.6	0.606	**9**	**1.6**	**<0.001****
Malig. Melan.	**5**	**2.1**	**0.045***	0	1.3	0.254	5	3.4	0.386
Prostate	1	1.5	0.683	**3**	**0.9**	**0.027***	4	2.4	0.302
Stomach	2	5	0.180	1	0.3	0.201	**3**	**0.8**	**0.014****

Observed and expected (Du Pont) counts reported in Table 1 of Chen *et al*. P-values calculated by OEHHA.
* $p \leq 0.05$; ** $p \leq 0.01$

DMF-Only Cohort: Poisson

Cancer Type	Wage			Salary			Total		
	Obs	Exp	p-value	Obs	Exp	p-value	Obs	Exp	p-value
All cancers	34	25.3	0.057	13	14.7	0.707	47	40	0.152
Bucc/pharynx	**8**	**1**	**<0.001****	1	0.6	0.451	**9**	**1.6**	**<0.001****
Malig. Melan.	5	2.1	0.062	0	1.3	1.0	5	3.4	0.256
Prostate	1	1.5	0.777	3	0.9	0.063	4	2.4	0.221
Stomach	2	5	0.960	1	0.3	0.259	**3**	**0.8**	**0.047***

Observed and expected (Du Pont) counts reported in Table 1 of Chen *et al*. P-values calculated by OEHHA.
* $p \leq 0.05$; ** $p \leq 0.01$

Epidemiological Studies Discussion

- **Limitations**
  - DMF exposure not quantified in Navy F4 or leather tanning workplaces
  - DMF production and use facilities:
    - Limited registry; cases *while employed*; limited number of cases
    - Truncated follow-up
    - Duration and intensity of DMF exposure not used in most analyses
    - Limited statistical power
    - Unable to reproduce analyses
- **Confounding exposures**
  - Workers exposed to many chemicals along with DMF in leather tanning and aircraft repair
  - Co-exposure to acrylonitrile not addressed in case-control study of production and use facilities

Epidemiological Studies Discussion

- **Exposure differences among industries may explain variable findings**
  - Higher levels of DMF in Navy F4 repair and leather tanning occupations likely
  - Dermal exposure associated with bioaccumulation of DMF (Chang *et al*., 2004, 2005)
  - Air levels experienced in production and use facilities were all fairly low (<10ppm average)

Epidemiological Conclusions

- Clusters of testicular germ cell tumors in two distinct occupationally exposed groups
- Case-control and cohort studies of leather tanners found an association of testicular germ cell tumors among workers exposed to DMF
- Some evidence of cancer risk among DMF production and use workers
- Definitive well-conducted studies needed

Carcinogenicity Studies in Animals

- **Drinking water study in rats** (Druckrey *et al*., 1967)
- **Two sets of inhalation studies in mice**
  - Male and female CD-1 mice exposed to 0, 25, 100, 400 ppm for 18 months (Malley *et al*., 1994).
  - Male and female BDF_1 mice exposed to 0, 200, 400, 800 ppm for 24 months (Senoh *et al*., 2004).

18 Month CD-1 Mouse Studies (Malley *et al*., 1994)

- No effect on survival in either sex.
- Body weights increased in both male and female for 400 ppm group.
- Increased liver to body weight ratios in 100 and 400 ppm males and 400 ppm females.
- Centrilobular hepatocellular hypertrophy and hepatic single cell necrosis at two highest doses (both sexes).
- No treatment related increase in tumor incidence ($p<0.05$).

24 Month BDF_1 Mouse Studies (Senoh *et al*., 2004)

- No effect on survival in either sex.
- Growth suppressed in exposed groups.
- Liver to body weight ratio increased with exposure in all exposed male and female mice.
- Centrilobular hypertrophy and nodules in exposed mice of both sexes.
- Hepatocellular adenomas and carcinomas increased in male and female exposed groups.

Liver Tumor Incidence in Male BDF_1 Mice (Senoh *et al*., 2004)

Tumor Type	Exposure Level (ppm)				Trend test[1]
	0	200	400	800	
Hepatocellular adenoma	6/50	36/50**	41/49**	41/50**	$p < 0.0001$
Hepatocellular carcinoma	2/50	12/50*	16/49**	16/50**	$p < 0.01$
Hepatoblastoma	0/50	13/50**	7/49*	4/50	$p = 0.464$
Combined	8/50	42/50**	46/49**	44/50**	$p < 0.0001$

[1] Exact test for linear trend
* $p<0.01$, pairwise comparison with controls by Fisher exact test.
** $p<0.001$, pairwise comparison with controls by Fisher exact test.

Liver Tumor Incidence in Female BDF_1 Mice (Senoh *et al*., 2004)

Tumor Type	Exposure Level (ppm)				Trend test[1]
	0	200	400	800	
Hepatocellular adenoma	1/49	42/50*	47/50*	48/49*	$p < 0.0001$
Hepatocellular carcinoma	3/49	25/50*	32/50*	35/49*	$p < 0.0001$
Hepatoblastoma	0/49	0/50	4/50	0/49	$p = 0.419$
Combined	3/49	45/50*	49/50*	49/49*	$p < 0.0001$

[1] Exact test for linear trend

* $p<0.001$, pairwise comparison with controls by Fisher exact test

Carcinogenicity Studies in Animals

- **Two sets of inhalation studies in rats**
  - Male and female CD rats exposed to 0, 25, 100, 400 ppm for 24 months (Malley *et al*., 1994).
  - Male and female F344 rats exposed to 0, 200, 400, 800 ppm for 24 months (Senoh *et al*., 2004).

24 Month CD Rat Studies (Malley *et al*., 1994)

- Survival not affected by DMF treatment.
- Body weights reduced in male rats exposed to 100 or 400 ppm; in female rats at 400 ppm.
- Relative liver weights increased in male and female rats exposed to 100 or 400 ppm.
- Centrilobular hepatocellular hypertrophy in all exposed groups of both sexes.
- No treatment related increase in tumor incidence ($p<0.05$).

24 Month F344 Rat Studies (Senoh *et al*., 2004)

- Survival unaffected in males
- Reduced survival in females exposed to 800 ppm due to liver necrosis.
- Body weights reduced in both sexes at 800 ppm dose.
- Increased liver to body weight ratios in rats of both sexes at all exposure levels.
- Centrilobular necrosis in both sexes at highest dose, but significant only in female rats.
- Hepatocellular adenomas and carcinomas increased in male and female rats.

Liver Tumor Incidence in Male F344 Rats (Senoh *et al*., 2004)

Tumor Type	Exposure Level (ppm)				Trend test[1]
	0	200	400	800	
Hepatocellular adenoma	1/50	3/50	13/50*	20/50*	$p < 0.0001$
Hepatocellular carcinoma	0/50	1/50	0/50	24/50*	$p < 0.0001$
Combined	1/50	4/50	13/50*	33/50*	$p < 0.0001$

[1] Exact test for linear trend
* $p<0.001$, pairwise comparison with controls by Fisher exact test

Liver Tumor Incidence in Female F344 Rats (Senoh *et al*., 2004)

Tumor Type	Exposure Level (ppm)				Trend test[1]
	0	200	400	800	
Hepatocellular adenoma	1/49	1/50	6/50	16/50**	$p < 0.0001$
Hepatocellular carcinoma	0/49	0/50	0/50	5/50*	$p < 0.001$
Combined	1/49	1/50	6/50	19/50**	$p < 0.0001$

[1] Exact test for linear trend
* $p<0.05$, pairwise comparison with controls by Fisher exact test
** $p<0.001$, pairwise comparison with controls by Fisher exact test

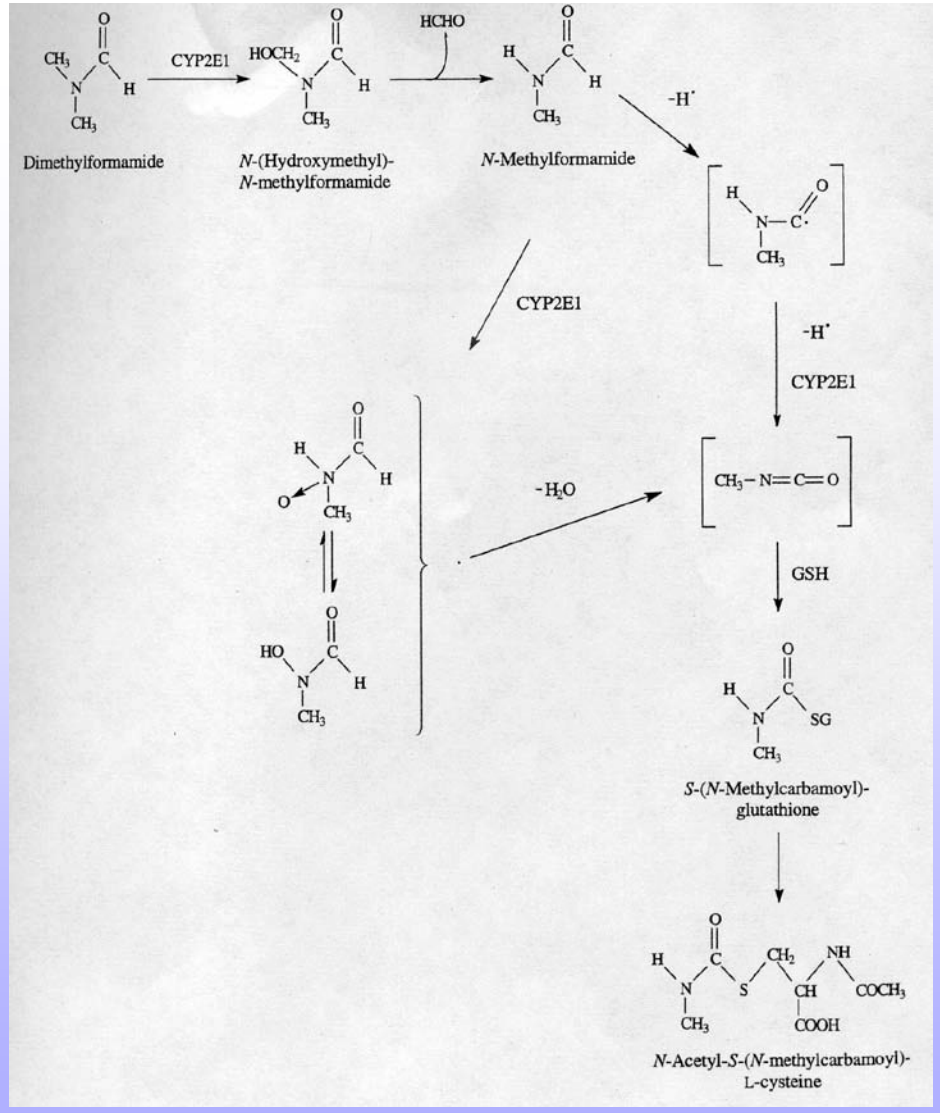
Conclusion of Carcinogenicity Studies in Animals

- **No tumors in drinking water study (Druckrey *et al.*, 1967).**
- **Hepatocellular adenomas and carcinomas increased with positive trend in male and female BDF_1 mice (Senoh *et al.*, 2004).**
- **Hepatocellular adenomas and carcinomas increased with positive trend in male and female F344 rats (Senoh *et al.*, 2004).**
- **No treatment-related tumors observed in studies in mice and rats by Malley *et al*. (1994) ($p<0.05$).**

Study Differences

- **Malley and Senoh studies differed in:**
  - Duration of mouse studies
    - Malley,18 months
    - Senoh, 24 months
  - Highest dose
    - Malley, 400 ppm
    - Senoh, 800 ppm
  - Strains
    - Mice: CD-1 (Malley), BDF_1 (Senoh)
    - Rats: CD (Malley), F344 (Senoh)

Metabolism of DMF



IARC, 1999

Other Relevant Data – Genotoxicity Data

- DMF is negative in most experimental systems from bacteria to mice (IARC, 1999).
- Some evidence of weak genotoxic activity from mouse lymphoma assay, UDS in rat hepatocytes, clastogenicity in yeast.

Other Relevant Data – Genotoxicity Data

- **Worker studies of chromosomal effects**
  - Chromosomal gaps and breaks in peripheral lymphocytes increased from 0.4% in controls to 1.4% in exposed workers (also exposed to methyl amines). (Berger *et al*., 1985)
  - Chromosomal aberrations increased in peripheral lymphocytes of workers exposed to DMF (also to trace amounts of other chemicals). (Koudela and Spazier, 1981)
  - SCEs increased significantly in high and medium DMF exposure groups of women workers (Seiji *et al*., 1992).

Other Relevant Data – Other Effects on Liver

- **Changes in liver to body weight ratios**
- **Histological changes**
  - Hypertrophy
  - Centrilobular necrosis
  - Altered cell foci

Some Possible Mechanisms of Action

- Genotoxicity
- Increased cell proliferation due to cytotoxicity or apoptosis
- Facilitates permeation of other chemicals

IARC Review

- Conducted in 1999 (before Senoh *et al*., 2004 studies)
- Humans: inadequate evidence of carcinogenicity
- Animals: suggested lack of carcinogenicity
- Group 3 – “not classifiable as to carcinogenicity in humans.”

Summary

- **Evidence of DMF Carcinogenicity**
  - Human studies:
    - Limited but suggestive evidence from occupational studies
  - Animals:
    - Hepatocellular adenomas and carcinomas were seen in male and female F344 rats.
    - Hepatocellular adenomas and carcinomas were seen in male and female BDF_1 mice (hepatoblastomas in males)
  - Other evidence:
    - DMF was at least weakly genotoxic in rodents and humans.