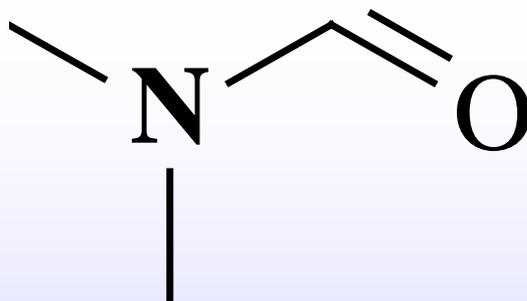


N,N-Dimethylformamide



Synonym: DMF

CAS No: 68-12-2



Physical-Chemical Data

- **Description:** colorless to slightly yellow liquid with faint amine odor
- **Molecular formula:** $\text{CHON}(\text{CH}_3)_2$
- **Molecular weight:** 73.09
- **Boiling point:** 153 C
- **Solubility:** Miscible with water and most common organic solvents
- **Conversion factor:** $\text{mg/m}^3 = 3.0 \text{ ppm}$
- **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 $p \leq 0.1$ cutoff**
 - Two-tail with $p \leq 0.1$ cutoff and one-tail with $p \leq 0.05$ 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 BDF₁ 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 BDF₁ 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 BDF₁ 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 BDF₁ 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 BDF₁ 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) ($p < 0.05$).

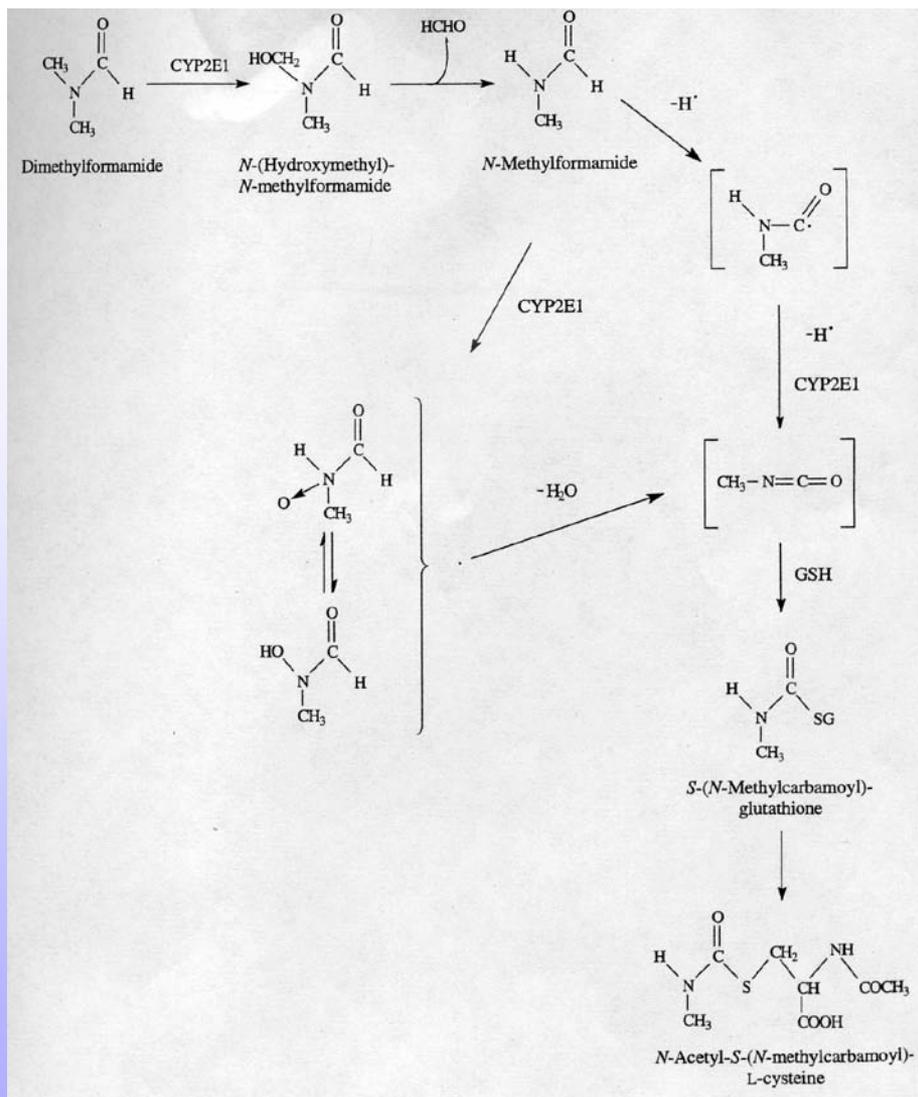


Study Differences

- **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), BDF₁ (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 BDF₁ mice (hepatoblastomas in males)
 - Other evidence:
 - DMF was at least weakly genotoxic in rodents and humans.

