EVIDENCE ON THE CARCINOGENICITY OF

N,N-Dimethylformamide

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee (CIC) of the OEHHA Science Advisory Board (Title 27 Cal. Code of Regs. §25301; formerly Title 22, Cal. Code of Regs. §12301).

On September 7, 2007 OEHHA announced in the *California Regulatory Notice Register* that N,N-dimethylformamide (DMF) was a chemical proposed for Committee consideration at their November 19, 2007 meeting. The September 7th notice also marked the start of a 60-day comment period during which interested parties could submit comments to OEHHA that would be forwarded to the members of the CIC prior to the November 2007 meeting. At their November 19, 2007 meeting, the Committee advised OEHHA to prepare hazard identification materials for DMF. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced on December 12, 2007, in the *California Regulatory Notice Register*. No information was received as a result of this request.

These hazard identification materials were complied to provide the Committee with relevant information for use in its deliberations. A public meeting of the Committee to discuss this evidence is scheduled for November 5, 2008. At this meeting it is expected that the Committee will render an opinion on whether DMF has been clearly shown to cause cancer. Written public comments should be submitted to OEHHA by October 7, 2008, in order to be considered by the Committee in advance of the meeting. During the November 5, 2008 meeting, the public will have an opportunity to present verbal comments to the Committee.

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1. EXECUTIVE SUMMARY

N,N-dimethylformamide (DMF) is a volatile solvent used in a variety of industries including acrylic fiber manufacture and styrene-butadiene rubber latex production (U.S. EPA, 2002; Gescher, 1993). It has also been used in aircraft maintenance, leather tanning, the production of plastics and pesticides, and the manufacture of adhesives, synthetic leathers, and surface coatings.

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed six studies of occupationally exposed workers and these studies provide some information on the potential for DMF to cause cancer, but all are limited in their ability to clearly elucidate this relationship. Well-conducted case-control (Frumin *et al*., 1989) and cohort studies (Calvert *et al*., 1990) of testicular cancer cases in tannery workers provide fairly compelling evidence that exposure to DMF, possibly in combination with other exposures, increased cancer risk in men in this occupational setting. The plausibility of this association is strengthened by a cluster of the same type of cancer in men exposed to DMF in an entirely different occupational setting (F4 aircraft repair, Ducatman *et al*., 1986), though no analytic study of these other workers was conducted.

In addition to the evidence from human studies, OEHHA has reviewed two sets of inhalation studies in male and female rats and two sets of inhalation studies in male and female mice. One set of studies conducted in each sex and species (reported in Senoh *et al.,* 2004) showed that DMF significantly increased the incidence of benign and malignant liver tumors in male and female rats and mice. The liver tumor incidences increased significantly with increased exposure level, and were increased especially at the two highest doses – 400 and 800 ppm. An earlier set of studies (reported in Malley *et al*., 1994), which included exposure up to 400 ppm, found no treatment-related increases in tumors in either sex or species, but observed DMF-induced liver toxicity (liver toxicity was also observed in the studies of Senoh *et al*., 2004).

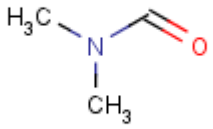
Genotoxicity studies indicate that DMF is at least weakly genotoxic in mammalian cells *in vitro* and provide suggestive evidence of genotoxicity in exposed humans. The carcinogenic activity of DMF in experimental animals and in humans may be due to a genotoxic mechanism, or DMF may have the ability to increase tissue-penetration of endogenous and exogenous carcinogens, or another as yet unknown mechanism, or a combination of mechanisms may be operative.

In summary, evidence from experimental animals indicates that DMF is carcinogenic in multiple species, inducing malignant and benign liver tumors in both sexes of rats and mice. Evidence from workers occupationally exposed to DMF, while limited, is nonetheless suggestive.

2. INTRODUCTION

2.1 Identity of N,N-Dimethylformamide

Molecular Formula:	$CHON(CH_3)_2$
Molecular Weight:	73.09
CAS Registry No.:	68-12-2
Chemical Class:	Amide
Synonym:	DMF, DMFA
Boiling point:	153° C



N,N-Dimethylformamide

2.2 Occurrence and Use

N,N-dimethylformamide (DMF) is a volatile solvent, albeit with a low evaporation rate. DMF is a high production volume chemical, primarily used as an industrial solvent (U.S. EPA, 2002). DMF solutions are used to process polymer fibers, films, and surface coatings; to permit easy spinning of acrylic fibers; to produce wire enamels, and as a crystallization medium in the pharmaceutical industry (US EPA, 1986). It has also been used in aircraft maintenance, leather tanning, the production of pesticides and plastics, and the manufacture of adhesives, synthetic leathers, and surface coatings (Gescher, 1993). During the late 1980s, between 94,000 (Frumin *et al*., 1989) and 100,000 (Levin *et al*.,1986) workers were exposed to DMF in the U.S. The U.S. Environmental Protection Agency (U.S. EPA, 2002) summarizes historical chemical production volume information on their web site and reports that 100 to 500 million pounds of DMF were imported or produced in the U.S. during the 2002 reporting year; this was two to ten times greater than the 50-100 million pounds produced or imported in the 1986 reporting year. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 18,249 pounds of DMF (CARB, 2000).

3. DATA ON N, N-DIMETHYLFORMAMIDE CARCINOGENICITY

3.1 Studies of Carcinogenicity in Humans

A body of human data was developed during the mid 1980's to the early 1990's addressing the possible association between the solvent DMF and cancer, in particular, testicular cancer.

Six human studies examining the relationship between DMF exposure and cancer were identified, including two cluster investigations, two case-control studies and two cohort studies. The studies are grouped by the type of exposure experienced by the study subjects, all of whom were occupationally exposed, and are described in chronological order, as the incidence of cancers among DMF-exposed workers was noticed or studied in different industries.

3.1.1 Navy F4 aircraft repairmen

A cluster investigation was performed after three male workers with testicular germ cell tumors were identified at one workplace (Ducatman *et al*., 1986). The men were employed at a Navy "aircraft exterior surface repair workplace" servicing F4 Phantom Jets and specifically using depotting agents containing DMF. The three men in the original cluster, among 153 white males at the workplace, met the case definition of "working at an airframe repair facility at least 3 years before the onset of signs or symptoms leading to a documented histopathological diagnosis of testicular germ cell cancer." They developed the tumors between 1981 and 1983. One additional man with a confirmed testicular germ cell tumor was found to be working elsewhere in the facility (out of 3,200 total employees, 2,450 being white males). He had a 20 year history with another F4 repair shop, but was not diagnosed with testicular cancer until his first year at the repair facility with the rest of the men in the cluster.

In response to this cluster at one F4 aircraft servicing facility (facility A), two other geographically distinct airframe repair shops were investigated for further cases (facilities B & C) (Ducatman *et al*., 1986). Only one of the two additional repair shops serviced the F4 (facility B). Facilities A and B are the only facilities used by the Navy for repairs on F4 aircraft. At facility B, four more individuals were identified who had a history of testicular germ cell cancers with onset from 1970-1983 (out of 680 white males) (Ducatman *et al*., 1986). At facility C, the repair shop that did not service the F4 Phantom Jets, none of the 446 white male employees had testicular germ cell cancers or a history of working on F4 airframe repairs. In contrast, all seven cases at facilities A and B had long histories of working on the exterior airframes of F4 aircraft (Ducatman *et al*., 1986). Questionnaires, interviews, and visits with workers, managers and medical facilities were carried out but did not reveal additional cases, including possible cases among the retired and deceased workers (Ducatman *et al*., 1986).

In considering what common exposure might have led to the finding of these clusters of cases, the authors noted that workers performing the repairs on the F4 aircraft at facilities

A and B were exposed to DMF via depotting agents, whereas workers at facility C were not exposed to depotting agents or DMF. Depotting is "performed by the electrical surface structure repairmen on the floor of the airframe repair area" and involves dripping "a solvent mixture containing 80 percent dimethylformamide…onto electrical cables without specific ventilation protection" (Ducatman *et al*., 1986). This procedure for depotting is unique to work on the F4 aircraft at these facilities. The maintenance work was completed near well ventilated hangers, though the workers could have had many shared industrial exposures from performing a variety of jobs. On other types of aircraft, such as those maintained in facility C, depotting is performed "on electrical assemblies that have been removed from the work area" (the authors provided no further information about the process). While all the repairmen were "exposed to many chemicals, metal dusts, paints, electroplated surfaces and solvents" the depotting solvent is, according to Ducatman *et al.* (1986), the only chemical exposure that could account for the clusters of testicular germ cell tumors at facilities A and B that were not present at facility C. Ducatman *et al*. (1986) concludes that the association between F4 airframe repair and testicular germ cell tumors is "highly suspicious and may represent a new sentinel event." A summary of the cluster investigations in aircraft repairmen and leather tanners is included in Table 1 below.

Table 1. Cases Included in Cluster Investigations of Testicular Germ Cell Tumors Among F4 Aircraft Repairmen and Leather Tanners.

Study author	Case no.	Industry/job	Year diagnosed	Estimated exposure level	Years exposed
Ducatman *et al*., 1986	1-3	F4 aircraft repairmen (facility A)	1981-1983	NR	4 - 19
	4-8	F4 aircraft repairmen (facility B)	1970-1983	NR	4 - 12
Levin *et al*., 1987	1	leather tanner (swabber)	1982	>10 ppm	13
	2	leather tanner (supervisor: swabber/cleaner)	1984	>10 ppm	14
	3	leather tanner (swabber)	1984	>10 ppm	8

NR – not reported

3.1.2 Leather tanners

Cluster investigation

Levin *et al.* (1987) reported a case series of testicular germ cell cancers in three leather tanners. The three men were "swabbers on the spray lines in the leather finishing process" at the same plant and developed histologically similar testicular cancer (embryonal cell carcinoma) after 8, 13 and 14 years of working as a swabber. The three

men, aged 25, 32, and 36 years, were diagnosed in 1982 or 1984 (see Table 1). Two of the men worked on the spray line full time. The swabbers worked with their heads close to the leather while leaning over the hide and using "felt bottomed paddles to spread the dyes." The third man supervised the line most of the day, working on the spray line a few hours a day, repairing the spray guns when necessary, and cleaning the spray guns daily. Windows were the only source of ventilation but were closed in winter. The National Institute for Occupational Safety and Health (NIOSH) visited the tannery and collected air samples, but DMF had been removed from the process in 1987 after the initial investigation of the cluster and was not detected in any samples (Frumin *et al*., 1989). Although DMF was later regulated by the Occupational Safety and Health Administration (OSHA) and a Permissible Exposure Limit (PEL) of 10 ppm was established. Frumin *et al*. (1989) speculated that the workers' exposure to DMF may have been greater than 10 ppm before DMF was removed from the process.

Case-control study at Fulton County tannery

Frumin *et al*. (1989) examined testicular cancer in leather tanners in Fulton County, New York, in a case-control study beginning with the same cluster of cases in the Levin *et al*. study described above. The employees in the cluster of three cases worked together on the night shift and over their course of their medical treatment, recognized their common malady and "subsequently brought the cluster of cases to the attention of investigators," including the workers' union, the New York State Department of Health, and Mount Sinai School of Medicine, as well as NIOSH. Additional cases were sought by Frumin in the New York State Cancer Registry, with cases classified as adult male residents of Fulton County aged 20 to 54 who had "developed testicular cancer between January 1974 and March 1987" (Frumin *et al*., 1989). Seven additional cases were identified by this process. The control group consisted of 129 Fulton County men who were in the same age range (race not specified) and developed another type of cancer during the same time frame. Five of the 10 cases (50%) and 17 of the 129 controls (13%) had been employed in "leather-related occupations." The resulting odds ratio for testicular cancer among those in leather-related occupations was statistically significant (Odds Ratio [OR]=5.8, 95% Confidence Interval [CI] 1.5-22.0) (Frumin *et al*., 1989).

Cohort study at Fulton County tannery

Calvert *et al.* (1990) conducted a cohort study of workers at the leather tannery where the testicular cancer cases first occurred (Levin *et al.*, 1987) and were included as part of the case-control study (Frumin *et al.*, 1989). Based on the company records, 80 workers had worked on the finishing line between 1975 and 1987. Person-years were calculated and the expected number of testicular cancers was estimated using "age- and calendar-year-specific incidence rates for New York State (excluding New York City)." The resulting incidence ratio (IR) was statistically significant and highly elevated for testicular cancer in tannery workers (IR= 40.5, 95% CI 8.1-118.4) (Frumin *et al*., 1989; Calvert *et al*., 1990) for testicular cancer in tannery workers.

Table 2. Analytic Studies of Testicular Cancer in Leather Tanners in Fulton County, NY.

Study author and type	Source of cases N	Comparison	Year diagnosed	Estimated DMF exposure level	Risk Ratio (95% CI)
Frumin *et al.*(1989) Case-control	Cancer registry plus cluster group 5 exposed[1] / 10 total	Listed in cancer registry, developed another type of cancer, during same time frame, matched on age 17 exposed / 129 total	Jan. 1974 – March 1987	>10 ppm	OR=5.8* (1.5 - 22.0)
Calvert *et al*. (1990) Cohort	Person-years at risk at tannery (total of 80 individuals) 1975 - 1987	Expected cancer incidence based on age- and calendar year-specific ratios for NY State (excluding NYC)	Jan. 1974 – March 1987	>10 ppm	IR= 40.5* (8.1 - 118.4)

* $p<0.05$
[1] "exposed" means employed in leather-related occupations

DMF production and use facilities

Two studies (Chen *et al*., 1988a, b; Walrath *et al*., 1989) were performed among facility employees at four Du Pont DMF production and use plants, described in the reports of these studies as Plants A through D. Plant A was a production facility which began as a pilot from 1938-1954 with commercial production commencing in 1961. In Plants B and C, DMF was "used as a spinning solvent in the manufacture of acrylic fiber." At Plant D, DMF was used as a "solvent for inks used to tint plastic sheeting." Cancer cases were obtained from the Du Pont Cancer Registry, which included only cancers diagnosed while a person was actively employed at Du Pont, and was limited to cancers diagnosed during or after 1956 (Chen *et al*., 1988a, b; Walrath *et al*., 1989). Individuals diagnosed with cancer after terminating employment were not included in the registry.

Table 3. DMF Exposure and Population Characteristics at the Du Pont Facilities (Chen *et al.*, 1988a; Walrath *et al.*, 1989).

Type of facility	**Plant A**	**Plant B**	**Plant C**	**Plant D**
	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	Evenly distributed between <1, 1 to <2, and 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

The cohort study by Chen *et al*. (1988a, b) included 2,530 employees from all four plants who were exposed to DMF but not acrylonitrile (ACN), a potential confounder, and 1329 employees who were exposed to both DMF and ACN, resulting in a DMF-exposed cohort of 3,859 employees. DMF exposure was classified into three categories, low (no direct contact and <10 ppm DMF in the air), medium (intermittent contact and more than once a week >10 ppm DMF in the air), and high (frequent contact and >10 ppm DMF in the air often) (Chen *et al*., 1988a). There were 1,130 employees exposed to neither DMF nor ACN in the cohort.

The cancer cases were extracted from the Du Pont Cancer Registry that began in 1956 and "contains cancer diagnosed while individuals were employed at Du Pont" (Chen *et al*., 1988a). Individuals diagnosed with cancer after leaving employment were not included in the cohort. Despite the presence of a set of unexposed employees who could serve as a comparison group, the authors do not provide any results from such comparisons. Observed cancer incidence was tabulated and expected cancer incidence was estimated using the Du Pont Company (1956-1984) and national (Surveillance Epidemiology and End Results [SEER]; National Cancer Institute) cancer incidence rates (1973-1977) (Chen *et al*., 1988a). The reason for choosing to use these particular years of SEER incidence rates was not provided. The authors compared the observed and expected cancer incidence rates but did not report p-values. OEHHA calculated the inherently one-tailed p-values using the chi-square (χ^2) distribution with one degree of freedom (comparison of point estimates). These results are provided in Table 4 below.

Because effects of exposure to ACN, a chemical known to the State of California to cause cancer (listed in 1987), cannot be untangled from any potential effects of DMF exposure in individuals exposed to both substances in this study, only results related to individuals

identified by the authors as exposed to DMF-only are discussed. OEHHA undertook additional analyses of the data presented in the Chen *et al.* (1988a, b) articles (see below).

In the DMF-only cohort, the total number of cancer cases was 47. The observed incidence (9 cases) of buccal cavity and pharynx cancer was elevated and statistically significant when compared against the Poisson expected rates for Du Pont (1.6 expected; p-value <0.0001) and SEER (3.3 expected; p-value=0.0017) (Chen *et al*., 1988a). Chen *et al*. (1988a) noted that all the exposed employees with buccal cavity and pharynx cancers (nine total) were found to be heavy smokers for at least 20 years and two were heavy drinkers. The observed incidence of malignant melanoma (five cases) was elevated and significant when compared against the Du Pont rates (2.1 expected; p-value = 0.045) and the SEER rates (1.6 expected; p-value = 0.0072), however the authors report that the relationship with the Du Pont rates was not significant for malignant melanoma (Chen *et al*., 1988a). According to Chen *et al*. (1988b), the liver is the expected toxicity target for DMF; however, no liver cancers were found by this study, although some were reported in the companion study of the same plants conducted by Walrath *et al.* (1989).

OEHHA analyses

OEHHA tested all the relationships reported by Chen *et al*. (1988a) using the chi-square distribution with one degree of freedom for the observed and expected counts of each cancer using the following relationship:

Null hypothesis (H_o): observed count = expected count

Alternative hypothesis (H_a): observed count ≠ expected count

$$Z^2 = \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \text{ and p-value} = P(\chi_1^2 \geq Z^2)$$

P-values were not reported in Chen *et al*. (1988a), but significance was reported at the 0.1, 0.05, or 0.01 levels. It appears that Chen *et al.* (1988a) mistakenly doubled every p-value in an attempt to provide a two-tailed p-value. However, the chi-square distribution is strictly positive and only has one tail. The chi-square distribution with one degree of freedom is the square of the normal distribution, so a one-tailed chi-square p-value is analogous to the two-tailed p-value from the normal. The chi-square p-value represents the probability of the observed counts being significantly different from expected in either direction and covers the entire alternative hypothesis, which is in qualitative terms inherently "two-tailed." Though Chen *et al*. (1988a) mentions the possibility of increasing the chance of finding a significant association by multiple testing, p-value adjustments were not addressed.

Tables 1 (DMF-only cohort compared to Du Pont rates) and Tables 3-5 (non-exposed cohort, DMF/ACN cohort and all DMF cohort, respectively, compared to Du Pont rates) from the Chen *et al.* (1985) publication, are reproduced in the Appendix with the correct chi-square p-values. The correct p-values result in the identification of 14 additional associations significant at the $p \leq 0.1$ level across the four cohorts. According to OEHHA's p-value calculations (see Appendix), in the DMF-only cohort, the cancer categories that are elevated and significant at the $p \leq 0.05$ level are: buccal/pharynx (wage, total), malignant melanoma (wage), prostate (salary), and stomach (total) and all

other cancer (total). When compared to the results in Chen *et al*. (1988a), there are three additional cancer categories in the OEHHA analysis that are statistically significant within the DMF-only exposure group (statistically different than expected, at the $p \leq 0.05$ level). In the non-exposed cohort, incidence of malignant melanoma (total) and thyroid gland (salary, total) cancers were statistically different (at the $p \leq 0.05$ level) than expected.

OEHHA then compared the cancer incidence between each exposure group and the non-exposed group using a chi-square analysis that assumes the probability of cancer in each of the exposed cohorts is identical to the non-exposed cohorts. Table 4 shows the cancer cases reported in Chen *et al*. (1988a) and notes which cancers are significantly higher in the exposed group when compared against the non-exposed group. The DMF-only cohort has a statistically increased proportion of cancer from the nonexposed group for the buccal cavity/pharynx, prostate, and lymphatic cancers. ($p \leq 0.05$)

Table 4. Increased Cancer Incidence in DMF-Only Exposed Cohort in Chen *et al.* (1988a) Cohort Study.[1]

Cancer type	Non-exposed	DMF Only	Comparison with non-exposed employees	Comparison with Du Pont[3] or SEER[4] rates
All cancers	17	47	p = 0.4	
Buccal cavity & pharynx	1	9	p = 0.012	Du Pont: p < 0.0001 SEER: p = 0.002
Lung	4	11	p = 0.7	
Melanoma	4	5	p = 0.5	Du Pont: p = 0.045 SEER: p= 0.007
Prostate	NA[2]	4	p = 0.011	
Stomach	NA[2]	3	p = 0.07	
Intestine	NA[2]	2	p = 0.3	
Nervous	NA[2]	3	p = 0.07	
All lymphatic/ Lymphohematopoietic	NA[2]	4	p = 0.011	
Bladder	NA[2]	2	p = 0.3	
All other	6	4	p = 0.2	
Total cohort size	1130	2530		

[1] Cancer counts reported in Chen *et al*. (1988a) and p-value calculated by OEHHA.
[2] Incidence was not reported in Chen *et al*. (1988a). OEHHA assumed omission implies no occurrence of these tumor types. An incidence of 0.25 was used to allow for test statistic calculation (a zero incidence creates and undefined test statistic).
[3] Expected rates based on cancer incidence in Du Pont employees (1956-1984).
[4] Expected rates based on U.S. cancer incidence rates from SEER (1973-1977).

Case-control study

To assess the effect of DMF exposure, Walrath *et al*. (1989) examined buccal cavity and pharynx, liver, testis and malignant melanoma cancers in a case-control study among employees at the four Du Pont production and use facilities described above. The cases were obtained from the Du Pont Cancer Registry of male employees diagnosed with cancer from 1956-1985. There were two controls for each case, matched by sex, whether they were hourly or salaried workers, birth year (± 3 years) and plant (A, B, C or D). Each employee was assigned two measures of exposure, average exposure and peak exposure (Walrath *et al*., 1989). Information on length of time employed or the number of years exposed to DMF was presented only categorically (e.g., <10 years duration of exposure vs. ≥10 years duration), despite the collection of information for each employee that would have allowed for analyses of these variables in a continuous manner. Also, since individuals who retired or otherwise left employment were lost to follow-up, the lack of significant findings in relation to latency or exposure duration is not surprising. In addition, although investigators were clearly aware of the presence of other exposures such as ACN in these plants, these exposures are not characterized in this study, i.e., cancer cases as well as controls may have had exposure to ACN and/or DMF.

Adjusted plant-specific odds ratios (ORs) displayed in Table 5 were calculated by Walrath *et al*. (1989) for each of the categories of cancer. For each cancer, the four plant-specific ORs are distinct from each other, suggesting that plant may be a confounder. Given the different types of activities carried out at the plants, and the reported variation in average exposure level (see Table 3), it is likely that the exposures may have been dissimilar, as was the proportion of employees exposed (see Table 3). In addition, the method by which employees were handling DMF could also have varied among the plants, but this is not discussed in either Walrath *et al*. (1989) or Chen *et al*. (1988a). A major concern with this study is that cases and controls were matched on plant, which appears to be a surrogate for exposure to some extent. This could have obscured any effect, if both cases and controls had similar exposures.

The adjusted ORs for the sites reported by Walrath *et al*. (1989) are shown in Table 5. Although ORs at Plant A, the DMF production plant, were elevated for several sites (buccal cavity and pharynx; malignant melanoma; prostate; testicular), none was statistically significant, and most were based on small numbers of cases. The only liver cancer OR reported by Walrath *et al*. (1989), for all plants combined, was elevated but not significant (OR=6.1; 90% CI = 0.38-72.0), with four of the six cases coming from Plant A.

For malignant melanoma, Walrath *et al*. (1989) reported a significant positive trend with increasing exposure using logistic regression ("present" [<1 ppm], OR = 0.85, 90% CI=0.19-3.80; "low" [1 to <2 ppm], 1.86, 90% CI= 0.47-7.34; "moderate" [2 to <10 ppm], 3.11 90% CI= 0.81-11.9). According to the authors, "none of the job titles at any of the plants fell into the highest …exposure rank [≥10 ppm]."

Prostate cancer cases in Plant D produced the only OR that was statistically significant (based on four cases: OR=8.04; 90% CI = 1.04, 62.3). According to Walrath *et al*. (1989), three of the four cases in Plant D were exposed to DMF 12 to16 years prior to onset of symptoms (two at levels < 0.1 ppm DMF and one at 2 to <10 ppm DMF). Given

that prostate cancer was significantly elevated in the Chen *et al.* (1988a) study of the workers in these plants, both in those workers exposed only to DMF ($p<0.05$, based on four cases) and those exposed to both ACN and DMF (data not shown; $p<0.01$), confounding by ACN exposure is especially a concern with regard to this finding.

Elevated testicular cancer at Plants A and C was not significant (based on four cases at Plant A, OR=15; 90% CI = 0.37-608; based on one case at Plant C, OR = 3; 90% CI = 0.11-80.5). The 11 cases of testicular cancer identified by Walrath *et al.* (1989) at the four plants contrasts with the Chen *et al.* (1988a) study at these same plants, which did not separately report cases of testicular cancer, having found only one case in the entire cohort.

Table 5. Cancer Cases Reported by Walrath *et al.* (1989) at DMF Production and Use Plants.

Cancer Type	Numerical Values	Plant A	Plant B	Plant C	Plant D	Combined
Buccal cavity & pharynx	OR[1] =	15	0.5	0.5	1.0	0.89
	90% CI =	(0.37-608)	(0.08-3.03)	(0.05-4.89)	(0.18-5.69)	(0.35-2.29)
	# Cases =	11	8	11	9	39
Liver	OR[1] =	NR	NR	NR	NR	6.1
	90% CI =	NR	NR	NR	NR	(0.38-72.0)
	# Cases =	4	0	0	2	6
Malignant melanoma	OR[1] =	3.5	1.0	1.02	2.0	1.7
	90% CI =	(0.45-27.5)	(0.10-10.2)	(0.14-7.32)	(0.12-32.6)	(0.52-5.51)
	# Cases =	9	6	11	12	38
Prostate	OR[1] =	2.0	1.43	0.4	**8.04**	1.47
	90% CI =	(0.20-19.6)	(1.04-62.3)	(0.07-2.28)	**(1.04-62.3)**	(0.66-3.30)
	# Cases =	16	10	13	4	43
Testicular	OR[1] =	15	0.33	3.0	0.33	0.99
	90% CI =	(0.37-608)	(0.01-8.93)	(0.11-80.5)	(0.03-3.41)	(0.22-4.44)
	# Cases =	4	2	1	4	11

[1] Mantel-Haenszel Odds ratio
NR: not reported
Bold indicates significant association at the 0.1 level

3.1.3 Discussion

Six studies of cancer in humans in relation to exposure to DMF were reviewed. These studies provide some information on the potential for DMF to cause cancer, but all are limited in their ability to clearly elucidate a relationship. Two cluster investigations (Ducatman *et al*., 1986; Levin *et al*., 1987) raised concerns about potential carcinogenicity in two different types of occupationally-exposed individuals, F4 aircraft repairmen and leather tanners, but the nature of these studies is such that they are primarily useful for generating hypotheses. Further investigations of the cluster of testicular cancer cases in tannery workers in Fulton County, New York in the form of well-conducted case-control and cohort studies by Frumin *et al*., (1989) and Calvert *et al*. (1990) provide fairly compelling evidence (OR=5.8, 95% CI 1.5 - 22.0; IR= 40.5, 95% CI 8.1 - 118.4) that exposure to DMF, possibly in combination with other exposures, results in increased cancer risk in men in this occupational setting (OR=5.8, 95% CI 1.5 - 22.0; IR= 40.5, 95% CI 8.1 - 118.4). Issues raised by these results including possible co-carcinogenicity posed by exposures to other chemicals, and the role of dermal exposure, deserve examination and are examined below. The final two studies by Chen *et al*. (1988a) and Walrath *et al*. (1989) present analyses of cancer incidence among employees at four Du Pont production and use plants, but both have serious methodological short-comings discussed in more detail below, that limit the ability of these studies to identify an effect of DMF exposure, if one exists. Nevertheless, results reported in these studies provide some indication of increased cancer risk in exposed plant employees. Given the small number of cancer cases included, most of the risk estimates do not achieve statistical significance. Some investigators, for example Chen and Kennedy (1988) and Gollins (1991), have characterized studies of Du Pont employees as providing evidence against DMF carcinogenicity. However, definitive well-conducted studies investigating the relationship between DMF exposure and cancer in humans have yet to be conducted, so this characterization is inaccurate.

Potential co-carcinogenicity or solvent effect

The possibility that co-exposures might explain the findings seen in aircraft repairmen and leather tanners was raised soon after the initial findings were published by Ducatman *et al.* (1986). In a letter to the editor, Chen and Kennedy (1988) comment that the "liver is the target for DMF toxicity" and question the carcinogenic effect found in the cluster of leather tanners, pointing to the cohort study they conducted (Chen *et al*., 1988a) on Du Pont employees which found no association between DMF exposure and testicular cancers. Ducatman (1989) responded to the comments with a letter to the editor hypothesizing that co-exposure to DMF and "heavy metal pigments, notably chromates" could explain the cluster of pathologically similar cases in the leather tanning and aircraft repair industries, noting that the latter were exposed to chromates present as dust from the grinding processes in F4 repair shops. Ducatman (1989) also suggested that DMF may allow for better dermal absorption of the carcinogenic chromates and concluded that the Du Pont employees have different exposures from the cluster cases. Du Pont employees were not known to have dermal contact, while the leather tanners and F4 repairmen had extensive dermal contact (Ducatman, 1989). Further follow-up on the issue of co-carcinogenesis in another letter to the editor by Gollins (1991) noted the dermal contact

in tannery and aircraft repairmen and stated that "DMF may simply be acting as a solvent which facilitates absorption through the skin of dissolved carcinogens."

Ducatman (1989) cited two studies of other occupational groups which evaluated exposure to solvents and testicular cancer, among other effects. These studies, though not relevant to DMF carcinogenicity *per se*, do support the hypothesis that concurrent exposure to solvents and chromates or other known carcinogens may increase testicular cancer incidence. Garland *et al.* (1988) studied U.S. Navy personnel who were involved in occupations of "aviation support equipment technician, engineman, and construction mechanic" and found they had higher rates of testicular cancer than those expected in the "US population and the total Navy population" (standardized incidence ratio (SIR) = 3.8, 95% CI = 1.9-5.6 for SEER and SIR = 3.8, 95% CI = 2.1-6.3 for Navy). The men performing these duties were exposed to degreasing agents and solvents, as well as fuels, oils, paints, and combustion exhaust emissions (Garland *et al.*, 1988). Guberan *et al.* (1989) examined the disability of painters and electricians in a cohort study and found increased cancer incidence in painters for several sites, including the testis (SIR=3.13, 90% CI 1.23-6.57). These authors noted that the painters were exposed to zinc chromate.

Importance of dermal exposure

Recent studies (Chang *et al*., 2005; Chang *et al*., 2004) have evaluated the importance of route of exposure to DMF in relation to its bioaccumulation. Chang *et al*. (2005) suggests that the biological half-life of DMF may be greater when exposure occurs dermally than via inhalation. These authors studied synthetic leather (SL) factory workers (frequently exposed to DMF liquids), copper laminate circuit board (CLCB) factory workers (rarely exposed to DMF liquids but exposed to DMF via inhalation) and controls matched on age and sex from other nearby factories without DMF exposure. Dermal and airborne (breathing zone samples) exposures were measured for all study subjects daily for five consecutive work days, and pre-shift urines were collected daily, and analyzed for DMF and the metabolite N-methylformamide. These investigators confirmed that the study control subjects had no airborne or dermal exposure to DMF. Breathing zone measurements were not statistically different between the SL and CLCB workers. Dermal levels of DMF were higher on the hands than forearms and significantly greater in SL workers than in CLCB workers. A linear accumulation in the levels of urinary DMF and N-methylformamide across the five days was observed in the SL workers but not in the CLCB workers. The authors concluded that the bioaccumulation of DMF observed in the SL workers was attributable to the increased dermal exposure experienced by these workers, as compared with the CLCB workers and was likely due to route specific differences in pharmacokinetics, i.e., absorption, distribution, metabolism, and elimination of DMF.

The potential for dermal exposure of Du Pont employees was not described by the investigators (Chen *et al*., 1988a; Walrath *et al*., 1989). A description of the assessment of exposure by Walrath *et al*. (1989) does not mention route, but provides estimates of average and 95th percentile air levels and metabolites in urine. The findings by Chang *et al*. (2005; 2004) indicate that increased bioaccumulation of DMF occurs in dermally exposed workers relative to inhalation-exposed workers. Extensive dermal exposure occurred in the tannery workers and aircraft repairmen (Ducatman 1989) compared to the

unknown dermal exposure of employees at the Du Pont plants. For these reasons the dose received by these different sets of exposed workers may be an important aspect to consider in evaluating the results found in these studies.

Limitations and findings in studies of Du Pont employees

The two studies of cancer in Du Pont employees working at four plants that produced or used DMF (Chen *et al*., 1988a, Walrath *et al*., 1989) both have serious methodological limitations, some that they share and others that are unique. Despite this, there are findings of interest in considering the potential for DMF to cause cancer.

One major limitation of both studies is the use of the Du Pont Cancer Registry, which includes only cases diagnosed among active employees, beginning in 1956 (Chen *et al*., 1988a, Walrath *et al*., 1989). Those who left for other employment, retirement or otherwise before being diagnosed were not included, limiting the potential to follow individuals for the length of time for cancer to develop, and to the age of life when cancers are mostly likely to occur. The limited registry also had a relatively small number of cases, given the number of employees working in these four plants during the time periods of interest. Another limitation related to the registry is that cases were noted beginning in 1956, however, the production plant began operation in 1938 and cases that occurred during those 16 years prior to 1956 were not tracked.

A second important limitation of both studies is the failure to adequately analyze the role of duration and intensity of DMF exposure in relation to cancer incidence. The studies' methods are flawed in different ways, as different approaches were used to assign and analyze exposure in these two studies, despite having been published just a year apart by some of the same investigators. Employees in these plants were assigned exposure levels using descriptive categories of relative exposure (low, moderate and high) by Chen *et al*. (1988a), citing the lack of monitoring data between 1950 and 1970, the limited period during which DMF-only employees were exposed, used descriptive categories of relative exposure (low, moderate and high). In these descriptions, both moderate and high level categories include air levels of DMF that were either "sometimes" (moderate) or "often" (high) greater than 10 ppm. In contrast, Walrath *et al*. (1989) based exposure assignment on data collected over the years on air measurements and metabolites in urine, with a measured geometric mean for the highest exposure group of greater than or equal to 10 ppm, but reported that none of the job titles of study subjects from any of the plants fell into this category. Both studies analyzed cancer cases diagnosed among active employees from 1956 to 1984 (Chen *et al*., 1988a) or 1985 (Walrath *et al*., 1989).

In analyzing the effect of exposure, the cohort study (Chen *et al*., 1988a) noted that workers paid hourly ("wage" workers) had a greater potential for chemical exposure than "salary" workers, and analyzed these two groups separately. Given the truncated follow-up inherent in the Du Pont Cancer Registry, this division of available cases into two categories of workers further reduced the number of cases of any specific cancer to less than five for most sites, meaning that analysis by exposure category (low, moderate, high) was without adequate power to distinguish a difference. The result is that analyses of cancer incidence in DMF-only exposed workers evaluated all exposure levels together, splitting cases into two categories based not on assigned exposure but on the wage/salary

category. Despite the presence of an unexposed portion of the cohort, comparisons conducted by the authors were done primarily in relation to "Du Pont Company rates" (1956-1984). As an example of what could have been evaluated instead, the publication provides data on the exposure levels experienced by cases of buccal cavity and pharynx cancers in DMF-only exposed workers. This cancer category had the greatest number of cases and was the only category that showed a significantly elevated incidence; six of the nine cases were highly exposed, and the other three moderately exposed, with a latency period from first exposure of more than 10 years, for all but one of these cases. The ability to assess this level of detail about exposure levels for other cancer categories would provide much better information on the possible effect of DMF on cancer incidence, but only limited information of this sort was included in the Chen *et al*. (1988a) study.

Despite the detailed exposure assessment exercise they undertook, Walrath *et al*. (1989) divided the results for each cancer type or category by the plant where employees worked, rather than by exposure level. Since cases were already matched to controls by plant and age, this grouping may have eliminated any observed differences based on exposure classification, given that Walrath *et al*. (1989) note that "DMF exposure patterns varied by plant." In addition, Walrath *et al.* (1989) failed to address the issue of potential co-exposure of the subjects in their study to ACN, a known carcinogenic substance present in these plants.

Finally, there are limitations of these studies with respect to their power and calculations. The statistics reported by Chen *et al*. (1988a) were calculated incorrectly, as discussed above. Chen *et al.* (1988a) doubled every p-value, in an apparent attempt to provide a two-tailed p-value. However, the chi-square distribution is strictly positive and only has one tail. This led to fewer significant associations (at the $p<0.1$ level) being reported than there should have been. Only a very small number of workers were included in the case-control study conducted by Walrath *et al*. (1989). As few as one case and two controls per plant and a maximum of 16 cases and 32 controls per plant were examined, making it difficult for any associations that might be found to reach statistical significance.

In light of all the limitations that reduce the ability of these studies to identify a relationship between exposure to DMF and cancer, a couple of the findings are of interest. Liver cancer was elevated in the Walrath *et al*. (1989) study, with all cases coming from the more-likely-to-be-exposed "wage" category, although based on Walrath *et al*.'s exposure classification scheme, only two of the cases and one of the controls were "exposed." All of the liver cancer cases met the standard for having at least 10 years latency from first exposure and greater than 10 years duration of exposure. With regard to testicular cancer, while Chen *et al*. (1988a) reported finding only one case, Walrath *et al*. (1989) found 11 cases, three of whom had DMF exposure, including two at the moderate exposure level, the highest level assigned to any subject in the Walrath *et al*. study. These two testicular cancer cases were both exposed to DMF for more than three years but less than 10. Walrath *et al*. (1989) did not perform any statistical analyses of the testicular cancer cases by exposure status as assigned based on monitoring data and job duties, nor did they describe the exposure status of the other cases or of the controls, except for noting that eight of the cases were from the two plants with the lowest frequencies and levels of DMF exposure (Plants A and D).

Summary

Well-conducted case-control (Frumin *et al*., 1989) and cohort studies (Calvert *et al*., 1990) of testicular cancer cases in tannery workers in Fulton County, New York provide fairly compelling evidence (OR=5.8, 95% CI 1.5 - 22.0; IR= 40.5, 95% CI 8.1 - 118.4) that exposure to DMF, possibly in combination with exposures to other chemicals, increased cancer risk in men in this occupational setting. The plausibility of this association is strengthened by a cluster of the same type of cancer in men exposed to DMF in an entirely different occupational setting (F4 aircraft repair, Ducatman *et al*., 1986), though no analytic study of these workers was conducted. Issues raised regarding the potential for co-carcinogenicity based on exposure to other substances (e.g., chromates) in both of these occupational settings cannot be dismissed. However, there is also recent data on differences in bioaccumulation of DMF depending on the route of exposure (Chang *et al*., 2005; Chang *et al*., 2004), with dermal exposure leading to greater dosing over time than inhalation exposure. This finding raises other possible explanations for the spike in cases seen in these two settings, both of which had substantial dermal exposure. The predominance of dermal exposure in these settings, together with the possibly higher air levels of DMF faced by the tannery workers as compared to employees at the Du Pont plants, may be part of the reason for the different strength of the findings seen in the former versus the latter occupational settings. The studies of employees at the Du Pont plants (Chen *et al*., 1988a, Walrath *et al*., 1989) have methodological limitations, including truncated follow-up, misclassification of exposure, and analytical errors that reduced their ability to find significant associations between DMF exposure and cancer incidence, should such associations exist. Despite these limitations, elevated, though not statistically significant risks were seen for liver cancer based on a small number of cases (OR=6.1; 90% CI = 0.38-72.0; Walrath *et al*., 1989). More definitive studies are needed, which accurately evaluate exposure to DMF (and other substances) over time, use appropriate comparison groups, and have more complete follow-up, in order to establish the carcinogenic potential of this chemical in humans.

3.2. Carcinogenicity Studies in Animals

A review of the scientific literature regarding carcinogenicity studies of DMF in experimental animals identified two long-term inhalation studies in male mice, two in female mice, two in male rats, and two in female rats.

3.2.1 Studies in mice

Malley et al. (1994) Mice (male and female) Crl:CD-1 (ICR) BR

Male and female mice (50 per group) were exposed to DMF by inhalation at 0, 25, 100 or 400 ppm concentrations in air for six hours per day, five days per week, for a period of 18 months. The high exposure concentration was chosen based on earlier toxicity experiments, and was expected to result in no significant life shortening. A full range of

tissues was collected from all animals after death or sacrifice, and examined using standard histological methods.

No compound-related effects on clinical observations or survival were observed in male or female mice. Body weights were increased for both male and female mice in the 400 ppm exposure groups. The 100 ppm and 400 ppm male mice and the 400 ppm female mice had increased liver weights as a percentage of body weight at the end of the 18 months. In the male mice the liver weights as a percentage of body weight increased from 5.85% in the controls to 7.06% and 7.80% in the 100 ppm and 400 ppm exposed mice respectively; in the female mice this value increased from 5.59% in the controls to 6.35% in the 400 ppm group (all statistically significant at $p<0.05$, by "pairwise comparison with controls" -- type of test not stated). Centrilobular hepatocellular hypertrophy and hepatic single cell necrosis were observed at increased frequency in mice of both sexes at the two highest doses. The incidence of centrilobular hepatocellular hypertrophy was 30 and 40% in the male and female mice exposed to 400 ppm DMF (statistically significant at $p<0.05$). The incidence of hepatic single cell necrosis was 30 and 18% in the male and female 400 ppm exposure groups, respectively (statistically significant at $p<0.05$).

No increased tumor incidence was observed in these studies.

Senoh et al. (2004) Mice (male and female) Crj:BDF$_1$ (SPF)

Male and female mice (50 per group) were exposed to DMF by inhalation at 0, 200, 400 or 800 ppm in air for six hours per day, five days per week, for a total of 24 months. The high exposure dose (800 ppm) was based on an earlier 13-week toxicity study. All animals received complete necropsy and histological examination of all major tissues.

There were no exposure-related effects on survival for any of the DMF-exposed mouse groups, but growth rates for all the exposed groups were suppressed in an exposure-dependent manner. Mice of both sexes exposed to DMF showed significantly increased liver weights in all exposed groups from 200 to 800 ppm. Male mice exposed to 200 ppm had relative liver weights of 11.0%; male mice exposed to 400 ppm had relative liver weights of 13.7% and male mice exposed to 800 ppm DMF had liver weights 17.8% of body weight compared to 3.9% of body weight in control males. Female mice exposed to 200 ppm had liver weights of 18.9%; female mice exposed to 400 ppm had relative liver weights of 25.8%; and female mice exposed to 800 ppm had relative liver weights 23.6% of body weight compared to 5.4% in control females. These increases were statistically significant at $p<0.01$ for all exposed groups of both sexes. Senoh *et al*. observed significant numbers of altered liver cell foci, which they characterized as "pre-neoplastic lesions" in exposed mice of both sexes. They also observed centrilobular hypertrophy, which they characterized as a non-neoplastic lesion, in exposed mice of both sexes. It is not clear whether the altered cell foci really are pre-neoplastic. White, brown or red nodules were seen in the livers of "almost all of the DMF-exposed groups of both sexes" (data not shown). It is not clear whether these nodules might be related to the formation of tumors.

The liver was the only organ that was clearly affected by exposure to DMF in the mice. Blood urea nitrogen (BUN) was slightly increased in the 400 and 800 ppm exposed male mice, and in all exposed female mice, indicating possible kidney damage as well.

Incidences of hepatocellular adenoma and carcinoma were statistically significantly increased in all DMF-exposed groups of male and female mice, compared to controls, and significant trends with dose were observed (Tables 6 and 7). Hepatoblastomas were statistically significantly increased above controls in male mice exposed to 200 ($p<0.001$) and 400 ppm DMF ($p<0.01$). The incidence of hepatoblastomas in the female mice exposed to 400 ppm (4/50) and in the male mice exposed to 800 ppm (4/50), while not statistically significant, exceeded the range of historical controls for male and female Crj:BDF_1(SPF) mice, respectively, observed in 18 sets of two-year inhalation carcinogenicity studies conducted in this laboratory (males: 2/897; females: 0/899). The combined incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma was statistically significantly increased ($p<0.001$) in all DMF exposed male and female mice.

Table 6. Liver Tumor Incidence in Male Crj:BDF_1(SPF) 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	$p = 0.464$
Liver tumors (Hepatocellular adenoma, carcinoma, and hepatoblastoma)	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.

Table 7. Liver Tumor Incidence in Female Crj:BDF$_1$(SPF)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	$p = 0.419$
Liver tumors (Hepatocellular adenoma, carcinoma, and hepatoblastoma)	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

3.2.2 Studies in rats

Malley et al. (1994) Rats (male and female) Crl:CD BR

Male and female rats (50 per group) were exposed by vapor inhalation to 0, 25, 100 or 400 ppm DMF in air for six hours per day, five days per week, for 24 months. The high exposure concentration was chosen based on earlier toxicity experiments, and was expected to result in no significant life shortening. A full range of tissues were collected from all animals after death or sacrifice, and were examined using standard histological methods. Survival in males and females was unaffected by treatment with DMF. Body weights were reduced in male rats exposed to 100 ppm DMF, and in male and female rats exposed to 400 ppm DMF. Male and female rats exposed to 400 ppm had significantly lower body weights compared to controls. The female rats exposed to 400 ppm appear to have an average body weight of approximately 400 grams at the end of the study, compared to approximately 500 grams for the non-exposed and lesser exposed female rats. At 500 days the male rats exposed to 400 ppm had an average body weight of approximately 700 grams compared to over 800 grams for the control rats. The male rats exposed to 100 ppm had their body weights reduced by a lesser amount – from approximately 800 to approximately 750 grams at the end of the experiment. According to the authors, "only the lower body weight and body weight gain observed in 400 ppm males and females and 100 ppm males were considered to be compound related."

Relative liver weights were increased in the 100 and 400 ppm exposure groups in both sexes. In the male rats the liver weights were increased from 2.87% of body weight in the controls to 3.58% in the 400 ppm exposed rats after 24 months (statistically significant at $p<0.05$). In the female rats the liver weights were increased from 3.12% to 3.86% in the 400 ppm exposed rats after 24 months (statistically significant at $p<0.05$). Likewise, the serum sorbital dehydrogenase activity levels were increased in the 100 and

400 ppm exposure groups of both sexes. Sorbital dehydrogenase activity is a sensitive indicator of hepatocellular injury (Malley *et al.,* 1994). In the male rats the activity levels increased from 2.0 units/liter in the controls to 9.7 units/liter in the 400 ppm exposed rats after 24 months. In the female rats the activity levels increased from 5.7 units/liter in the controls to 12.9 units/liter in the 400 ppm exposed rats after 24 months (statistically significant at $p<0.05$).

Centrilobular hepatocellular hypertrophy was seen in livers of all exposure groups of both sexes, increasing in a dose-dependent manner. This effect was minimal in the 25 ppm exposure group. Lipofuscin/hemosiderin accumulation in Kupffer cells was also observed in all exposure groups of both sexes. Centrilobular single-cell necrosis was seen only in the 400 ppm exposure group in both males and females.

No statistically significant increase in tumors was observed in any exposure group in these studies.

Senoh et al. (2004) Rats (male and female) F344/DuCrj (SPF)

Male and female rats (50 per group) were exposed by inhalation to 0, 200, 400 or 800 ppm DMF vapor in air for six hours per day, five days per week, for 24 months. The high exposure dose (800 ppm) was based on an earlier 13-week toxicity study. All animals received complete necropsy and histological examination of all major tissues.

Body weights were reduced (statistically significant at $p<0.05$) in both sexes of rats at the highest concentration (800 ppm). Survival was unaffected in the male rats but was reduced in the female rats exposed to 800 ppm to 30/50 (60%) compared to 42/49 (86%) in the control female rats, due to centrilobular necrosis of the liver. Mean body weight of male rats exposed to 800 ppm DMF was 299 grams compared to 393 grams for the control rats. Mean body weight of female rats exposed to 800 ppm DMF was 196 grams compared to 277 grams for control rats. Increased liver weights were also observed in both sexes of rats at all exposure levels (200 to 800 ppm) compared to the controls (statistically significant at $p<0.05$). In male rats the liver weights increased from 3.1 to 5.7% of body weight; in female rats the liver weights increased from 2.7 to 5.0% of body weight. Altered liver cell foci occurred in a statistically significant dose-dependent manner in rats of both sexes. Centrilobular necrosis occurred at the highest dose in rats of both sexes, but was statistically significant only in the female rats.

The incidence of hepatocellular adenomas was increased in male rats in the two highest exposure groups, to 13/50 in the 400 ppm group ($p<0.001$) and 20/50 in the 800 ppm group ($p<0.001$) compared to 1/50 in the control group (Table 8). The incidence of hepatocellular carcinomas was increased in male rats to 24/50 in the 800 ppm exposure group compared to 0/50 in the control group (significant at $p<0.001$). In female rats there were statistically significant increases in the incidences of hepatocellular adenomas and carcinomas in the 800 ppm exposure group (adenoma: 16/50, $p<0.001$; carcinoma: 5/50, $p<0.05$) compared to the incidences in controls (adenoma: 1/49, carcinoma: 0/49) (Table 9). The incidence of hepatocellular adenoma in the female rats exposed to 400 ppm DMF

was 6/50; while not statistically significant, this exceeded the historical range for control female rats in the laboratory (12/898, data from 18 two-year carcinogenicity studies).

Table 8. Liver Tumor Incidence in Male F344/DuCrj (SPF) 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
Liver tumors (Hepatocellular adenoma, and carcinoma)	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

Table 9. Liver Tumor Incidence in Female F344/DuCrj (SPF) 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
Liver tumors (Hepatocellular adenoma, and carcinoma)	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

3.2.3 Discussion of carcinogenicity studies in animals

DMF was toxic to the liver in both rats and mice in both the Malley *et al*. and the Senoh *et al*. studies. Both groups of investigators observed increased liver weights, centrilobular necrosis and or hypertrophy, histopathological changes (nodules) and changes in liver enzymes in both rats and mice. These effects were dose-related. From these observations it is clear that DMF enters liver cells in rodents and exerts toxic effects. It is not clear whether these toxic effects are related in some causal way to the tumors which were observed in the Senoh *et al*. but not the Malley *et al*. experiments.

With regard to tumor formation, there is an obvious contrast between the findings of the inhalation studies in male and female rats and mice of Malley *et al*. (1994) and those of Senoh *et al*. (2004). DMF induced statistically significant increases in liver tumors in male and female rats and mice in the studies of Senoh *et al*. (2004), whereas no treatment-related tumors were observed in the studies in rats or mice by Malley *et al.*(1994). The differences may be partly explained by the longer duration of exposure of the mice in the Senoh *et al*. studies (24 months versus 18 months in the Malley *et al*. studies), the higher top dose employed in the Senoh *et al*. studies (800 ppm versus 400 ppm in the Malley *et al*. studies), and the different strains of mice and rats used. With regard to the duration of the mouse studies, it is important to note that the liver tumors did not begin killing the mice in the Senoh *et al*. studies until after 18 months, which was the full duration of the Malley *et al*. mouse studies. This suggests the possibility that the 18-month study duration employed in the mouse studies of Malley *et al*. was not long enough to observe DMF-induced tumors. With regard to the higher top dose used in the studies of Senoh *et al.*, this alone seems an unlikely explanation for the differences in tumor findings in mice, since increases in liver tumors in males and females were observed in the studies of Senoh *et al.* at doses equivalent to or one half the top dose employed in the studies of Malley *et al*. In the case of rats, however, there could be a question about whether the high dose exceeded the maximum tolerated dose (MTD). According to the U.S. EPA's criteria for MTD, the high dose should not cause a significant increase in mortality. In the female rats the MTD was exceeded because of the high mortality brought about by centrilobular liver necrosis.

The histopathology evaluations of the animals in the Malley *et al*. studies appears to have been just as thorough as those in the Senoh *et al*. studies, so this does not appear to have been a factor that would explain the difference in results.

3.3. Other Relevant Data

3.3.1 Absorption, distribution, metabolism and excretion

Experimental systems

The absorption, distribution, metabolism and excretion of DMF have been studied in a wide range of experimental systems including rats, mice, hamsters, monkeys and *in vitro* systems with human liver microsomes (IARC, 1999). Studies in rats, mice and cynomolgus monkeys exposed to DMF in air showed that the compound was rapidly taken up into the bloodstream and then rapidly eliminated. Plasma half-lives in the monkeys were 1-2 hours for DMF, and 4 to 15 hours for the metabolite N-methylformamide (Hundley *et al.*, 1993). No information was provided about tissue distribution in these animals.

All of these studies indicate the involvement of similar metabolic pathways in different species, but the accumulation of different intermediates in the bloodstream and the rates of excretion differ across species.

In all systems studied, DMF is first metabolized by cytochrome P450 2E1 to N-hydroxymethyl-N-methylformamide (HMMF) (Gescher, 1993; Mraz and Nohova, 1992b; IARC, 1999). This intermediate has been widely used as a biological exposure index for DMF (Mraz and Nohova, 1992b). Metabolism continues through a series of intermediates to S-(N-methylcarbamoyl)glutathione (SMG) and eventually (through several intermediates) to N-acetyl-S-(N-methylcarbamoyl)-L-cysteine (AMCC). AMCC is a major metabolite in humans, but not in rodents (Mraz and Nohova, 1992b). In humans exposed to DMF by inhalation, AMCC accumulates in the blood and tissues and is relatively slowly excreted over a period of more than 48 hours (Mraz and Nohova, 1992b). The greater importance of the AMCC pathway in humans compared to rodents may be significant in terms of the biological effects (toxicity and carcinogenicity) to humans (Mraz and Nohova, 1992b). It is not known at this time whether the most important reactive intermediate is AMCC itself, or one of the metabolites leading up to it (Mraz and Nohova, 1992b; IARC, 1999). All we can conclude at this time is that because of the species differences in metabolism and excretion of DMF and its metabolites, one might expect differences between rodents and humans in the biological endpoints caused by DMF exposure, including carcinogenicity. This expectation seems to be confirmed by the fact that DMF exposure produces liver tumors in rats and mice, and is associated with increases in testicular tumors in exposed humans. Not all steps in the metabolism of DMF are established with certainty; there may also be other minor pathways. DMF metabolism is an area of active research; for reviews see Gescher (1993), Mraz and Nohova (1992b), and IARC (1999).

Humans

Humans who are exposed to DMF vapor may absorb DMF into their system by both the dermal and inhalation routes (Mraz and Nohova, 1992a,b). Studies on volunteers who were exposed to DMF vapor in the atmosphere at a concentration of 50 mg/m^3 for four hours showed that percutaneously absorbed DMF accounted for 13 to 36% of urinary HMMF, a major metabolite of DMF in humans (Mraz and Nohova, 1992a).

Volunteers (five men, five women) were placed in atmospheres of 10, 30, and 60 mg/m^3 DMF for eight hours. Urine was collected, and metabolites measured for up to five days (Mraz and Nohova, 1992b). Additionally, two men and two women were exposed to 30 mg/m^3 for eight hours per day on five consecutive days. The uptake from the respiratory tract was 90%, and urinary metabolites accounted for 49% of the retained dose (Mraz and Nohova, 1992b). The metabolite that was retained the longest was AMCC, with a half-life of 23 hours, compared to two hours for DMF, four hours for HMMF, and seven hours for N-hydroxymethylformamide (Mraz and Nohova, 1992b).

Metabolites found in urine of workers exposed to DMF have been evaluated in a number of studies reviewed by International Agency for Research on Cancer (IARC, 1999). All of these showed that HMMF in urine correlates well with the amount of DMF to which workers are exposed during an eight-hour shift, making HMMF a useful biomarker for worker exposure. Two studies (Sakai *et al*., 1995; Casal Lareo and Perbellini, 1995) also

showed that AMCC is a good measure of total exposure to DMF over a prolonged period, owing to its slower rate of excretion than the other metabolites (IARC, 1999). As discussed above, two recent studies (Chang *et al*. 2004; Chang *et al*., 2005) measured excretion of DMF and N-methylformamide (an earlier product of DMF metabolism than HMMF, and also considered to be a good bioindicator of DMF exposure) in the urine of synthetic leather and copper laminate circuit board (CLCB) workers after one week of DMF exposure. The authors determined that the synthetic leather workers, with exposure via both dermal and inhalation routes had more bioaccumulation of DMF over the five-day period than the CLCB workers with primarily inhalation exposures. The authors suggested that route specific differences in pharmacokinetics may have accounted for the increased bioaccumulation of DMF observed in the synthetic leather workers.

3.3.2 Genotoxicity data

Genotoxicity studies have been reviewed by the International Agency for Research on Cancer (IARC, 1999), and are summarized below. No more recent genotoxicity data has been found in literature searches.

Experimental systems

In general, DMF has produced negative results when tested for genotoxicity in experimental systems ranging from bacteria to mice (IARC, 1999). In almost all of the reported studies DMF did not induce gene mutations in any strain of *Salmonella typhimurium* or in *Escherichia coli* (IARC, 1999). DMF did, however, induce a slight increase in unscheduled DNA synthesis (UDS) in primary rat hepatocytes in one study, but not in two other studies with mouse and Syrian hamster hepatocytes (IARC, 1999). DMF did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*, but it did increase mutations frequency about two-fold in mouse lymphoma cells (IARC, 1999). Two studies in *Saccharomyces cerevisiae*, one of aneuploidy (Parry and Sharp, 1981), the other of homozygosis (Zimmerman and Scheel, 1981), indicate clastogenic activity of DMF in this system (reviewed by IARC, 1999). DMF has not been assayed adequately for DNA damage (no COMET assay) nor for oxidative DNA damage. In summary, there is some evidence of weak genotoxic activity from the mouse lymphoma assay, the UDS findings in rat hepatocytes, and clastogenicity assays in yeast.

Humans

One major difficulty in evaluating the genetic toxicity of DMF in humans is that almost all the available data are derived from workers who are exposed to other chemicals along with DMF, making it difficult to discern the effects of DMF alone. In one study in Germany, chromosomal gaps and breaks were seen in 1.4% of workers exposed to a mean value of 12.3 mg/m^3 DMF, while the frequency in unexposed control workers was only 0.4% (Berger *et al*., 1985, reported in IARC, 1999). This appears to be a moderately positive result. However, the exposed workers were concomitantly exposed to lesser

amounts of two other chemicals: monomethylformamide and dimethylamine, making this result difficult to interpret.

A study of chromosome aberration frequencies in about 40 workers in Czechoslovakia was reported by Koudela and Spazier (1981) and reviewed by IARC (1999). Blood samples were obtained from the workers during two four-month periods when they were exposed to 180 and 150 mg/m^3 DMF. At the same time they were exposed to trace amounts of other organic chemicals including 2-butanone (methyl ethyl ketone), butyl acetate, toluene, cyclohexanone and xylenes. During these high-exposure periods the frequencies of chromosomal aberrations observed in the workers' blood were 3.82 and 2.75% respectively. Later, during three six-month periods of lower exposure (50, 40 and 35 mg/m^3) the chromosomal aberration frequencies dropped to 1.59, 1.58 and 1.49%, not far above the frequencies observed in two control groups (1.61 and 1.10%). Again, this appears to be a positive result during the periods of high exposure, but we have the confounding problem of concomitant exposure to other organic chemicals which could be adding to the chromosomal aberrations either independently or through synergistic action together with the DMF exposure.

The effect of occupational exposure to DMF on sister chromatid exchanges (SCEs) in peripheral lymphocytes of women was studied by Seiji *et al*. (1992) and reviewed by IARC (1999). Women exposed to higher levels of DMF (17.4 and 2.1 mg/m^3) had higher frequencies of SCEs per cell (8.26 and 7.24) than matched (control) women (5.63 and 4.66). Women exposed to a lower level of DMF (0.9 mg/m^3) did not have higher SCE frequencies than matched controls. The women who were exposed to the middle level of DMF (2.1 mg/m^3 or 0.7 ppm) were also exposed to 0.9 ppm toluene. Thus, there is a risk of confounding in the findings for the workers exposed to the middle level of DMF, but not as far as we can tell from the published report, for the workers classified as exposed to the highest levels of DMF.

In summary, there are three published reports reviewed by IARC of genotoxicity studies in workers. All three indicate some genotoxic activity, but there may be some confounding by concomitant exposures to other chemicals in all of the studies with the exception of the women exposed to the highest level of DMF in the Seiji *et al*. (1992) study. Taken together these provide suggestive evidence that DMF may be genotoxic in some occupationally exposed humans.

3.3.3 Pathology

Changes in relative liver weights and histological changes such as formation of nodules or centrilobular hypertrophy were observed in treated groups of both sexes of rats and mice in both the Malley *et al.* and Senoh *et al.* studies. This indicates that DMF possesses hepatotoxic activity in rodents of both species and both sexes. The histological changes are described somewhat differently in the Malley *et al*. report and the Senoh *et al*. reports. Malley *et al.* describe "individual hepatocellular necrosis (apoptosis)" occurring in mice of both sexes for all exposed groups. OEHHA notes that apoptosis and

necrosis are usually considered very distinct mechanisms leading to cell death; Malley *et al*. seem to be conflating the two mechanisms. Malley *et al*. describe centrilobular single-cell necrosis and hypertrophy occurring in both male and female rats. Senoh *et al*. describe centrilobular hypertrophy occurring in exposed rats and mice of both sexes. This hypertrophy may be due to compensation by surviving cells to liver cell loss.

3.3.4 Mechanism of action

IARC concluded that DMF "does not appear to be genotoxic as judged from results of a variety of *in vitro* and *in vivo* assays" (IARC, 1999). However, as discussed above, there is some evidence of weak genotoxic activity of DMF from *in vitro* studies (e.g., mouse lymphoma assay, UDS assay in rat hepatocytes), and from occupational studies.
Since DMF is highly hepatotoxic in all experimental animal species examined (mouse, rat, nonhuman primates, etc.) it is possible that the mechanism of action that leads to hepatocarcinogenicity is a mechanism related to cell injury or cell killing, such as an apoptotic mechanism. Mechanisms that could cause germinal cell testicular tumors in humans are unknown. More research is needed to elucidate DMF's mechanism of action in inducing liver tumors in animals and the mechanisms associated with induction of germinal cell testicular tumors in humans.

As discussed above, it has been suggested that DMF's ability to act as a "permeation enhancer," *i.e*. to penetrate tissues, and act as a carrier for other chemicals (Olivella *et al*., 2007), may be involved in its mechanism of action. Either the DMF itself is carcinogenic, or it may facilitate entry of other (perhaps endogenous) carcinogens either through the skin or from the bloodstream or both.

The mechanism by which DMF induces cancer remains unclear. There is some data indicating that DMF is weakly genotoxic. Other mechanistic hypotheses have not been investigated or tested experimentally, and thus remain speculative.

4. OTHER REVIEWS

4.1 IARC

IARC (1999) found that there was *inadequate evidence* of carcinogenicity of DMF in humans, and evidence suggesting a lack of carcinogenicity of DMF in experimental animals (rodents). IARC's overall evaluation of DMF was "not classifiable as to its carcinogenicity in humans" (Group 3).

However, IARC produced its review in 1999 before the studies of Senoh *et al*. (2004) were available. The positive findings of Senoh *et al*. (2004) contradict those of Malley *et al*. (1994) and provide evidence of the carcinogenicity of DMF in male and female rats and mice.

4.2 Other agencies

No reviews were found from other agencies.

5. SUMMARY AND CONCLUSIONS

5.1 Summary of Evidence

The existing evidence demonstrates that DMF is clearly hepatotoxic and hepatocarcinogenic in rats and mice of both sexes. Case-control and cohort studies of exposed workers provide fairly compelling evidence that exposure to DMF, possibly in combination with other exposures, increases cancer risk in occupationally exposed men. DMF has been shown to be weakly genotoxic in some *in vitro* systems. There is suggestive evidence of genotoxic activity in humans exposed occupationally.

DMF induced liver tumors in some rodent studies (Senoh *et al*., 2004), but not in others (Malley *et al*., 1994). This apparent conflict of results may be due to differences in the animal strains used, differences in dosing regimens, and possibly other methodological factors.

The genotoxicity data reviewed above suggests that DMF may possess some weak genotoxic activity. Two of the possible hypotheses to explain the carcinogenic activity of DMF in rodents and humans would involve either its genotoxicity or its permeation-enhancing activity. There is some indication that DMF has the capacity to permeate through tissues (Olivella *et al*., 2007). This may enable DMF to reach target tissues and exercise its genotoxic activity, or DMF may act as an escort to facilitate the easy entry of either endogenous or exogenous carcinogens. The occupationally exposed workers were simultaneously exposed to other carcinogens such as metal salts. In the rodent studies the DMF may have increased penetration of endogenous carcinogens. These hypotheses are not mutually exclusive, nor are they the only possible hypotheses to explain DMF's apparent carcinogenic activity in rodents and humans. Only further research can clarify the mode of action by which DMF is able to cause tumors in experimental animals and exposed human beings.

5.2 Conclusion

The available evidence indicates that exposure of humans to DMF, in the aircraft maintenance and leather tannery industries, is associated with the development of testicular (germinal cell) tumors. Occupational studies of Du Pont workers when analyzed by OEHHA provided some evidence that DMF exposure increased cancer risk among these workers. These occupational studies have limitations due to the small number of cases, limited ability to track cases, failure to analyze the relationship between degree of exposure and outcome, and low statistical power and sometimes incorrect

statistical analysis. Other epidemiological studies (case-control studies) supplement the evidence from the occupational studies.

Long-term studies in mice and rats indicate that DMF exposure is associated with development of liver tumors. Investigators in both the human and animal arenas have discussed the possibility that DMF may act through either a direct carcinogenic mechanism, or by increasing the tissue-penetrating potential of other endogenous or exogenous carcinogens.

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7. APPENDIX

Table A1: DMF-Only Cohort with Du Pont Rates[1]

	Wage			Salary			Total		
Cancer Type	Obs	Exp	p-value	Obs	Exp	p-value	Obs	Exp	p-value
All cancers	34	25.3	0.08*	13	14.7	0.66	47	40	0.27
Buccal/pharynx	8	1	<0.001***	1	0.6	0.61	9	1.6	<0.001***
Lung	7	5.5	0.52	4	2.8	0.47	1	8.3	0.35
Malignant melanoma	5	2.1	0.05**	0	1.3	0.25	5	3.4	0.39
Prostate	1	1.5	0.68	3	0.9	0.03**	4	2.4	0.30
Stomach	2	5	0.18	1	0.3	0.20	3	0.8	0.01**
Intestine	2	2.4	0.80	0	1.9	0.17	2	4.3	0.27
Nervous system	2	1	0.32	1	0.5	0.48	3	1.5	0.22
All lymphohematopoietic	4	3.3	0.70	0	1.8	0.18	4	5.1	0.63
Bladder	1	1.3	0.79	1	0.7	0.72	2	2	1.00
All other	2	6.7	0.07*	2	3.9	0.34	4	10.6	0.04**

[1]Observed and expected counts as reported in Table 1 of Chen *et al*. (1988a). P-values computed by OEHHA. See text for details.
* $p \leq 0.1$
** $p \leq 0.05$
*** $p \leq 0.01$

Table A2: Nonexposed Cohort with Du Pont Rates[1]

	Wage			Salary			Total		
Cancer Type	Obs	Exp	p-value	Obs	Exp	p-value	Obs	Exp	p-value
All	5	5.1	0.96	12	10.8	0.72	17	15.9	0.78
Buccal/pharynx	0	0.2	0.65	1	0.4	0.34	1	0.6	0.61
Lung	2	0.9	0.25	2	2	1.00	4	2.9	0.52
Melanoma	2	0.6	0.07*	2	0.9	0.25	4	1.5	0.04**
Thyroid	0	0.1	0.75	2	0.1	<0.001***	2	0.2	<0.001***
All other	1	3.3	0.21	5	7.4	0.38	6	10.7	0.15

[1]Observed and expected counts as reported in Table 3 of Chen *et al*. (1988a). P-values computed by OEHHA. See text for details.
* $p \leq 0.1$,
** $p \leq 0.05$
*** $p \leq 0.01$

Table A3: DMF/ACN Cohort with Du Pont Rates[1]

	Wage			Salary			Total		
Cancer Type	Obs	Exp	p-value	Obs	Exp	p-value	Obs	Exp	p-value
All	36	33.5	0.666	5	6.3	0.605	41	39.8	0.849
Buccal/pharynx	1	1.4	0.735	1	0.2	0.074	2	1.6	0.752
Digestive	4	7.4	0.211	1	1.6	0.635	5	9	0.182
Lung	10	8.1	0.504	0	1.3	0.254	10	9.4	0.845
Melanoma	2	2.2	0.893	0	0.5	0.480	2	2.7	0.670
Bladder	1	1.8	0.551	2	0.3	0.002***	3	2.1	0.535
Prostate	6	2.3	0.015**	0	0.4	0.527	6	2.7	0.045**
All lymphatic	6	3.7	0.232	1	0.7	0.720	7	4.4	0.215
All others	6	6.6	0.815	0	1.3	0.254	6	7.9	0.499

[1]Observed and expected counts as reported in Table 4 of Chen *et al*. (1988a). P-values computed by OEHHA. See text for details.

* $p \leq 0.1$

** $p \leq 0.05$

*** $p \leq 0.01$

Table A4: All DMF Cohort with Du Pont and SEER Rates[1]

	Wage					Salary					Total				
		Du Pont		Seer			Du Pont		Seer			Du Pont		Seer	
Cancer	Obs	Exp	p-value	Exp	p-value	Obs	Exp	p-value	Exp	p-value	Obs	Exp	p-value	Exp	p-value
All	70	58.8	0.14	68.6	0.14	18	21	0.14	27.5	0.14*	88	79.8	0.14	96.1	0.14
Buccal/pharynx	9	2.4	<0.001***	4.7	0.00**	2	0.8	0.00	1.9	0.00	11	3.2	<0.001***	6.6	0.00*
Lung	17	13.6	0.36	15.2	0.36	4	4.1	0.36	6.5	0.36	21	17.7	0.36	21.7	0.36
Melanoma	7	4.3	0.19	3.4	0.19*	0	1.8	0.19	1.1	0.19	7	6.1	0.19	4.5	0.19
Prostate	7	3.8	0.10	3.4	0.10*	3	1.3	0.10	1.8	0.10	10	5.1	0.10**	5.2	0.10**

[1]Observed and expected counts as reported in Table 5 of Chen *et al*. (1988a). P-values computed by OEHHA. See text for details.

* $p \leq 0.1$

** $p \leq 0.05$

*** $p \leq 0.01$