Dimethylformamide (DMF)

Reasons Why DMF Should Not be Listed as a Carcinogen Under Proposition 65

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Agenda

- Standard for Listing Under Proposition 65

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- Discussion of Animal Studies

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- Discussion of Epidemiology Data

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Standard for Listing Under Proposition 65

Statute: **"A chemical is known . . . to cause cancer if in the opinion of the state's qualified experts it has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer . ."**

Standard for Listing Under Proposition 65

Duty of CIC:

- **"Render an opinion . . . as to whether specific chemicals have been *clearly shown*, through *scientifically valid testing* according to *generally accepted principles*, to cause cancer."**

  Cal. Code Regs., tit. 22, § 12305(a)(1).

Standard for Listing Under Proposition 65

Chemical should be listed only

"if the

- ***weight of the evidence***
- ***clearly shows* that [it]**
- ***causes* invasive cancer**

in humans or . . . in animals . . ."

Guidance Criteria at 1.D.

Discussion of Animal Studies

Robust Toxicology Database

- Acute, subchronic, chronic, oncogenicity studies in rats, mice, hamsters, cats, dogs, monkeys
- Developmental toxicity studies in rats and rabbits
- Reproduction study in rats
- Metabolism/Pharmacokinetics: rats, mice, monkeys, humans
- Genotoxicity: *in vitro* and *in vivo*
- Epidemiology

Carcinogenicity Studies

- Two studies
  - Malley *et al.* 1994 & Senoh *et al.* 2004
  - Both used the inhalation route
  - Both used rats and mice
  - Both studies identified liver as the target organ
- Different results at purportedly overlapping exposure concentrations
- Differences in chamber atmosphere generation appear to have resulted in much higher systemic doses in Senoh *et al.*
- MTD exceeded in Senoh *et al.* 2004 due to higher concentrations and aerosol deposition on animals

Maximum Tolerated Dose

- OECD/EPA Guidelines for carcinogenicity studies require achieving an MTD, without greatly exceeding the MTD
  - The high dose should produce some toxic effects without producing significant adverse effects on overall health of the test animals
- Evidence of exceeding the MTD includes:
  - Significant decrease in body weight gain,
  - Significant changes in clinical chemistry,
  - Saturation of detoxification mechanisms,
  - Marked changes in organ weight, morphology, and histopathology
- Excessive dosing can compromise biological interpretation
  - Saturation of absorption and detoxification mechanisms can result in tumor formation that is secondary to cytotoxicity
  - Cancer observed only at doses exceeding the MTD does not “clearly show” that the test substance is a carcinogen

Senoh *et al.* Carcinogenicity Studies

Carcinogenicity and Chronic Toxicity after Inhalation Exposure of Rats and Mice to N,N-Dimethylformamide

Senoh *et al.*; J. Occupational Health 2004 Japan Bioassay Research Center, Japan

- *Animals:*
  - F344/DuCrj (SPF) rats: 50/group
  - Crj:BDF1 mice: 50/group
- *Exposures:*
  - 6 hr/day, 5 days/wk, 104 weeks
- *Concentrations*;
  - 0, 200, 400, 800 ppm

Male Mice: Senoh *et al.*

Parameters	0 ppm	200 ppm	400 ppm	800 ppm
Body weight (% of control)	-	87	78	70
Relative liver weight	3.9	11.0*	13.7*	17.8*
Serum LDH	415	4559**	3497**	2115**
Hepatocellular necrosis (single cell)	12/50	38/50**	43/49**	48/50**
Centrilobular nuclear atypia	0/50	33/50**	42/49**	45/50**
Hepatocellular adenomas	6/50	36/50*	41/49*	41/50*
Hepatocellular carcinomas	2/50	12/50*	16/49*	16/50*

Female Mice: Senoh *et al.*

Parameters	0 ppm	200 ppm	400 ppm	800 ppm
Body weight (% of control)	-	100	95	81
Relative liver weight (%)	5.4	18.9*	25.8*	23.6*
Serum LDH	847	3588**	6452**	3299**
Hepatocellular necrosis (single cell)	22/49	13/50	6/50**	19/49
Centrilobular nuclear atypia	2/49	7/50	3/50	16/49**
Hepatocellular adenomas	6/49	36/50*	41/50*	41/49*
Hepatocellular carcinomas	2/49	12/50*	16/50*	16/49*

Summary of Effects in Mice: Senoh *et al.*

- Non-linear dose response in key end points
  - Serum enzyme activity
  - Tumor incidence
  - Non-neoplastic and pre-neoplastic changes
- The severe impact on liver function demonstrates that MTD was exceeded at 200 ppm and above

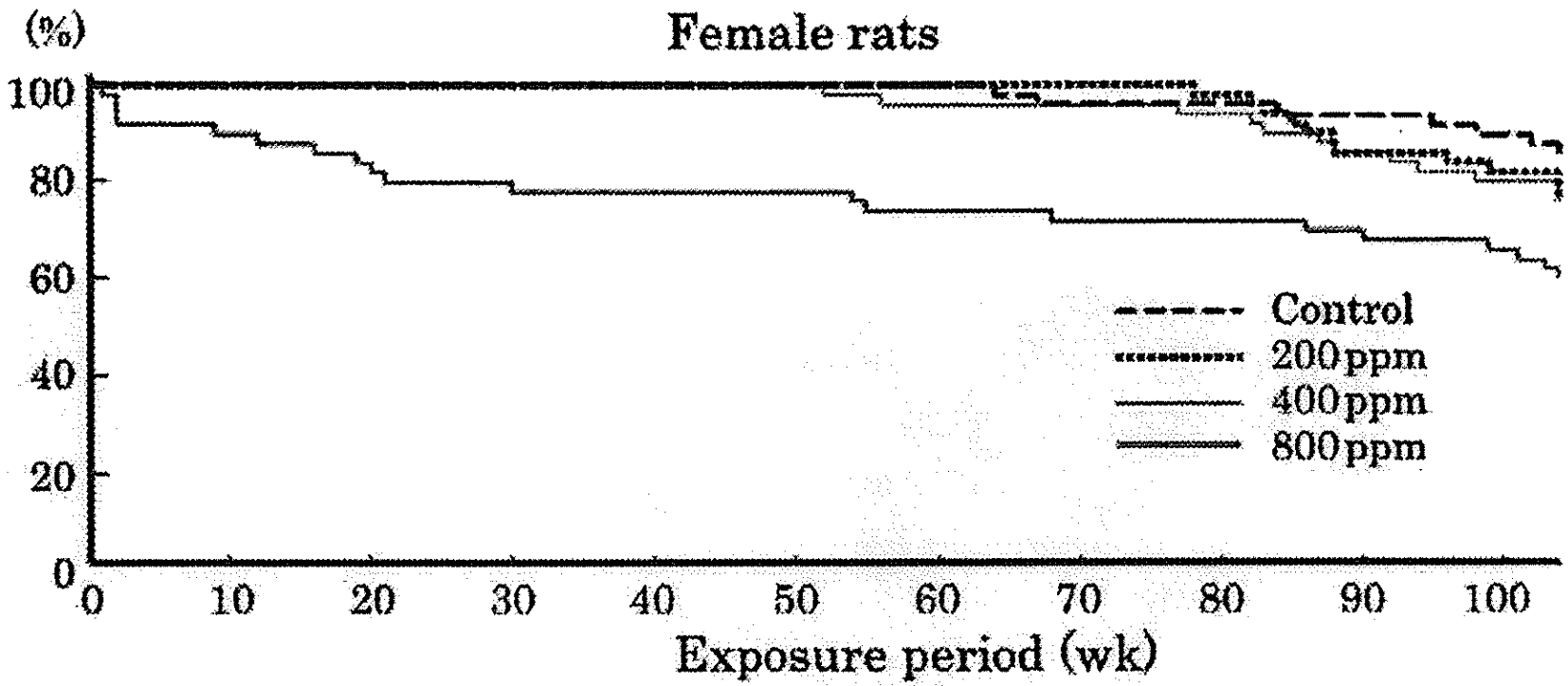
Male Rats: Senoh *et al.*

Parameters	0 ppm	200 ppm	400 ppm	800 ppm
Body weight (% of control)	-	93	87	76
Relative liver weight (%)	3.1	4.0*	3.8*	5.7*
Serum ALT	32	56**	96**	195**
Spongiosis hepatis	4/50	21/50**	26/50**	24/50**
Centrilobular necrosis	1/50	5/50	0/50	5/50
Hepatocellular adenomas	1/50	3/50	13/50**	20/50**
Hepatocellular carcinomas	0/50	1/50	0/50	24/50**

Female Rats: Senoh *et al.*

Parameters	0 ppm	200 ppm	400 ppm	800 ppm
Body weight (% of control)	-	92	77	71
Relative liver weight (%)	2.7	3.3*	3.7*	5.0*
Serum ALT	56	81**	79*	110**
Spongiosis hepatis	0/49	0/50	0/50	2/50
Centrilobular necrosis	0/49	3/50	2/50	13/50**
Hepatocellular adenomas	1/49	1/50	6/50	16/50*
Hepatocellular carcinomas	0/49	0/50	0/50	5/50*

Senoh *et al.* rat survival



Summary of Effects in Rats: Senoh *et al.*

- Increased mortality in 800 ppm females
- Decreased body weight at 400 and 800 ppm
- Increased hepatic tumors in 400 ppm and above males and in 800 ppm females
- Dose related increase in hepatic enzyme activity in males and females at 200 ppm and above
- Mortality, excessive body weight effects, and severe impact on liver function and pathology demonstrates that MTD was exceeded at 400 ppm and above

Malley *et al.* Carcinogenicity Studies

Chronic Toxicity/Oncogenicity of Dimethylformamide in Rats and Mice Following Inhalation Exposure

Malley *et al.*; Fundamental and Applied Toxicology 1994
DuPont Haskell Global Centers for Toxicology and Environmental Sciences

- ***Animals:***
  - **Crl: CD BR rats: 87/group**
  - **Crl: mice: 78/group**
- ***Exposures:***
  - **6 hr/day, 5 days/wk, 78 weeks (mice) or 104 weeks (rats)**
- ***Concentrations;***
  - **0, 25, 100, 400 ppm**

Male Mice: Malley *et al.*

Parameters	0 ppm	25 ppm	100 ppm	400 ppm
Body weight (% of control)	-	99	103	103
Relative liver weight	5.85	5.94	7.06*	7.80*
Hepatocellular necrosis (single cell)	15/60	37/62*	43/60*	55/59*
Hepatocellular adenomas	13/60	11/62	11/60	11/59
Hepatocellular carcinomas	0/60	1/62	4/60	2/59

Female Mice: Malley *et al.*

Parameters	0 ppm	25 ppm	100 ppm	400 ppm
Body weight (% of control)	-	102	102	102
Relative liver weight	5.59	5.71	5.99	6.35*
Hepatocellular necrosis (single cell)	18/63	28/63*	44/61*	48/63*
Hepatocellular adenomas	0/63	1/63	2/61	1/63
Hepatocellular carcinomas	0/63	0/63	0/61	0/63

Summary of Effects in Mice: Malley *et al.*

- Increased relative liver weight at 100 ppm and above
- Increased incidences of non-neoplastic microscopic changes in the liver at 25 ppm and above
- Achieved but did not exceed MTD
- No increase in neoplastic lesions

Male Rats: Malley *et al.*

Parameters	0 ppm	25 ppm	100 ppm	400 ppm
Body weight (% of control)	-	101	91	86
Relative liver weight (%)	2.87	2.81	3.28	3.58*
Serum SDH	2.0	4.4*	18.3*	9.7*
Hepatocellular single cell necrosis	1/57	1/59	2/58	18/60*
Hepatocellular adenomas	1/57	1/59	3/58	2/60
Hepatocellular carcinomas	0/57	0/59	0/58	1/60

Female Rats: Malley *et al.*

Parameters	0 ppm	25 ppm	100 ppm	400 ppm
Body weight (% of control)	-	92	90	76
Relative liver weight (%)	3.12	3.43	3.33	3.86*
Serum SDH	5.7	9.0	4.9	12.9
Hepatocellular single cell necrosis	0/60	0/60	3/59	11/62*
Hepatocellular adenomas	0/60	1/60	1/59	0/62
Hepatocellular carcinomas	0/60	0/60	0/59	0/62

Summary of Effects in Rats: Malley *et al.*

- Decreased body weight at 400 ppm
- Decreased body weight in 100 ppm females
- Minimally increased SDH activity at 25 ppm and above males only (ALT, AST, LDH not increased)
- Increased incidences of non-neoplastic microscopic changes in the liver at 400 ppm
- Achieved but did not exceed MTD
- No increase in neoplastic lesions

Similarities Between Senoh *et al.* and Malley *et al.*

Route of exposure:	**inhalation**
Exposure frequency:	**6 hr/day, 5 days/week**
Exposure Mode:	**whole body**
Species:	**rat/mouse**
Duration (rats):	**24 months**

Differences Between Senoh *et al.* and Malley *et al.*

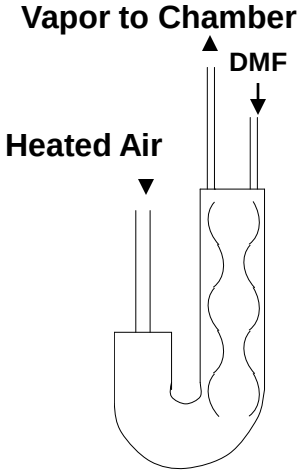
- Study duration for mice: 18 vs. 24 months
  - NTP requested 18 month duration as per EPA TSCA guidelines
- Method of atmosphere generation
- Exposure concentrations
  (dose selection and MTD considerations)
- Rodent strains

Method of Atmosphere Generation

Vapor pressure = 2.6 mm Hg @ 20ºC (Propensity for vapor condensation)

Malley *et al.* used methods to ensure vapor instead of aerosol

- Heated air
- Heated tubing
- Chamber airflow = 12 air changes/hr (as per guidelines)
- Aerosol not analytically detected



Senoh *et al*. methods likely resulted in aerosol generation

- "Spraying liquid DMF into air space of solvent chamber" (suggests possible aerosol presence)
- Chamber airflow = 6 air changes/hr
  - Low airflow rate promotes aerosol formation
  - Below guideline specifications
- GC sampling would not detect presence of aerosol in chamber

Method of Atmosphere Generation *(cont'd)*

- Delivered dose in the Senoh study is most likely greater than measured air concentration
  - Oral and dermal exposure from aerosol deposition on fur and skin likely significant
  - DMF has a high dermal absorption rate - contributing substantially to systemic toxicity
- The non-linear tumor response and response of serum enzyme activity observed in mice is consistent with exposure to a greater amount of DMF than reported
- Therefore, the dose to the animals in the Senoh study cannot be determined

OECD/EPA/NRC Guidelines for Dose Selection

- Dose selection should take into consideration
  - "Known or suspected nonlinearities of inflection points in the dose response"
  - "Pharmacokinetics and dose ranges where metabolic induction, saturation, or nonlinearity between external and internal doses does or does not occur"
  - The high dose should produce "some toxic effects without unduly affecting mortality …or producing significant adverse effects on nutrition and health of test animals"
- Signs of treatment-related toxicity associated with an excessively high dose includes
  - "Greater than 10% reduction in body weight gain"
  - "Significant changes in hematology or clinical chemistry parameters"
  - "Saturation of absorption and detoxification pathways"
  - "Marked changes in organ weight, morphology, histopathology"

Exposure Concentrations

- Evidence of excessive toxicity in Senoh studies include:
  - Excessive mortality in female rats
  - >20% change in body weight
  - Flat dose response for tumor incidence and hepatic enzyme activity in mice
- Effects are suggestive of metabolic saturation as well as exceeding the MTD

DMF Metabolism

```
     O   CH3                 O   CH2-OH              O   H
     ‖    |                  ‖    |                  ‖    |
H — C — N — CH3 →       H — C — N — CH3 →       H — C — N — CH3 → → undentified reactive metabolite

      DMF                   DMF-OH                  NMF                ↓
                                                                 glutathione conjugate (SMG)
                                                                       ↓
                                                   NHCOCH3          O
                                                      |             ‖
                                                    CH — CH2— S— C — N — CH3
                                                      |                  |
                                                    COOH               CH3   AMCC
```

- Metabolic pathway same in humans, primates, and rodents (Gesher, 1993)
- At high concentrations DMF inhibits its own biotransformation (WHO, 2001)

Metabolism is Saturated in Rats and Mice

Species	Exposure status	Exposure (ppm)	DMF µM*hour per ppm	Ratio
Rat	Single	250	42.8	
		500	175.1	4.1
	Repeat	250	37.6	
		500	59.0	1.6
Mouse	Single	250	17.2	
		500	249.0	14.5
	Repeat	250	6.6	
		500	59.1	8.9

Hundley *et al.*, 1993

Tumors observed in rats and mice occur at concentrations where metabolic pathways saturated

Rodent Strains

- Crl:CD-1 (ICR)(BR) mouse [Malley *et al.* study]
  - CD-1 inbred strain most commonly used in oncogenicity studies
  - "ICR" designation indicates international standard strain (ensures genetic similarity from global suppliers)
- Crj:BDF1 hybrid mouse [Senoh *et al.* study]
  - Hybrid of C57BL/6 and DBA strains
  - Uncommon strain
  - BDF1 mouse had different genetic mutations in hepatocellular tumors compared to other strains
  - Hybrid mouse strains typically used for animal disease/therapeutic models
  - Uncertain response of this hybrid strain to known carcinogens/non-carcinogens
  - Applicability of this strain for risk assessment is not clear
- OECD guidelines for animal selection in chronic toxicity/oncogenicity studies states that animals should be "commonly used laboratory strains"

DMF is Not Genotoxic

- DMF has been used in many genotoxicity assays
  - Negative in approximately 60 in vitro studies using bacterial and yeast strains, mammalian derived cell lines, and insect derived somatic cell lines
  - Negative in approximately 20 in vivo mammalian and insect assays
  - Positive in 6 in vitro studies
- Evaluation by IARC (1999):

  "Dimethylformamide has been extensively tested in a broad range of in-vitro and in-vivo genotoxicity assays. Results have been consistently negative in <u>well controlled studies</u>."

Animal Data Summary

- Liver is the target organ
- DMF has only been shown to induce hepatic tumors in situations of metabolic saturation and severe hepatocellular cytotoxicity
- Not genotoxic

Discussion of Human Data

Studies of DMF Carcinogenicity in Humans

3 groups of epidemiologic studies

1. Cluster study in F-4 aircraft repairmen
2. Cluster report, case-control study, and comparative incidence analysis among leather tannery workers in Upstate NY
3. Cohort study (Camden, SC) and case-control study of 4 plants among DuPont employees

Central questions

1. Is the review, reanalysis, and interpretation by OEHHA of the human data correct?
2. Do the human data support listing under Prop 65?

Comparison of Occupational Exposure Assessments

Study	Testicular Cancer Cases and Study Populations	Reported DMF Exposure Assessments
Aircraft Repairmen:	7 cases among 1279 white males at 3 repair facilities	Not reported: Depotting solution solvent mixture with 80% DMF used at 2 sites
Leather Workers: Cluster	3 cases at Pan American Tannery (PAT)	Tannery used DMF, but no levels reported for identified cases
Case-Control	10 cases / 144 controls with other cancers	“DMF was not detected by NIOSH in any air or bulk samples”
Comparative Incidence	3 cases among 83 total workers at PAT	Not detected in 20 air samples, usage discontinued in 1987
DuPont: Cohort	1 case among 5005 workers at Camden, SC site	Exposure classification committee: Low, Moderate, High (> 10 ppm)
Case-Control	138 cancer cases and 276 non-cancer controls at 4 sites including Camden, SC	Job title and work area estimates using > 8500 personal and air samples: Average, Peak, and Duration based on WH records

Cluster Studies

- Initial cluster report, Ducatman *et al.* (1986)
  - Hypothesis of association between testicular cancer and DMF arrived at after eliminating other candidate risk factors
  - "Our investigation raises, but does not prove, a hypothesis of association between [testicular cancer and exposure to a mixture containing DMF]."
- Leather tannery cluster report, Levin *et al.* (1987)
  - Letter to the editor of Lancet describing 3 cases at the Pan American Tannery
  - "…DMF, which became the focus of concern in light of the report by Ducatman *et al.* in 1986,…"

Limitations of the Cluster Studies

- **Small number of cases provides little statistical power to assess relationships for any occupational exposure**
- **No comparative analyses within each study population**
- **No documentation of DMF exposure levels or consideration of other chemical agents in the workplace**
- **No reports of symptoms consistent with increased DMF exposure including ‘flush’, alcohol intolerance, and liver disease (Redlich *et al.* 1988)**

Tannery Worker Case-Control Study

Reported in three documents:

1. **State of NY DOH report (1988)**
2. **Published version in MMWR (Frumin *et al.* 1989)**
3. **International Journal of Epidemiology (Marshall *et al.* 1990)**

Lack of any exposure estimates for DMF

- **No longer used at index facility at time of study**
- **No personal or area samples recorded during usage**

No assessment of exposure to other chemicals

- **Includes metals, synthetic dyes, and glycol ethers**

Tannery Worker Case-Control Study

Potential strong biases due to design and methods

- **Selection bias led to different age distributions**
- **Information bias for exposure classification: full work histories for cases, most recent occupation only for controls**

Inference from odds ratio

- **Exposure is defined as ‘ever working’ at a leather tannery**
- **This job assignment does not comprise only DMF exposure**

Tannery Worker Case-Control Study: Reported Results

	Cases	Controls with Occupation	Controls w/out Occupation
Number	10	115	29
Mean Age at Cancer Dx	31.7 yrs	47.0 yrs	41.3 yrs
20-29 yrs	4	7	5
30-39 yrs	5	9	7
40-49 yrs	1	39	8
50-54 yrs	0	60	9

Tables 1 and 4 State of NY DOH 1988 report

Tannery Worker Case-Control Study: Reported Results

	Cases	Controls with Occupation
Leather Work	**5**	**17**
No Leather Work	**5**	**98**
Odds Ratio = 5.76 (1.51, 22.07) *Controls missing exposure = 29*		

Table 3 from State of NY DOH 1988 report

Tannery Worker Case-Control Study: Revised Results

	Cases	Controls *
Leather Work	**5**	**32**
No Leather Work	**5**	**112**
Revised Odds Ratio = 3.50 (0.95, 12.85) **Controls assigned to leather work = 15 exposed, 14 not exposed*		

Revised Odds Ratio assuming 50% of missing controls are exposed to 'leather work'

Pan American Tannery Comparative Incidence Study

- Analytic result is SIR = 40.5 (95% CI: 8.2, 118.5) estimated for 3 observed cases and 0.07 expected cases at the Pan American Tannery
- Based on rates for Upstate NY from 1974 to 1985, there were 19 observed cases and 25.7 expected cases (SIR = 0.74, 95% CI: 0.4, 1.1)
- No evidence of high DMF exposure from medical screening of 51 workers out of 83 total at PAT
- "Based on these findings from the medical evaluation, it is unlikely that overexposure to DMF occurred at the tannery".

Conclusions from Comparative Incidence Study

- **Calvert *et al.* (November,1990) letter to the Lancet:**

  "This investigation confirms an excess of testicular cancer at a tannery. This adds to concerns about the carcinogenicity of DMF but conclusions should be tempered by a lack of detailed information about exposure to DMF and because of coexistent exposures to other chemicals at the tannery."

- **NIOSH report (Calvert and colleagues, January 1990):**

  "Because of the large number of chemicals at the tannery, the changes in engineering controls, the changes in chemical inventory over time and the absence of written records to document the changes in the chemical inventory, identification of the agent responsible for the testicular cancer cluster at the Pan American Tannery is impossible."

DuPont: Chen *et al.* Cohort Study

- Camden, SC acrylic fiber plant (identified as Plant C in Walrath study)
- 5,005 workers with 3,859 having exposure to DMF
- One case of testicular cancer in the cohort
- Main finding was 11 cases of buccal/pharynx cancer
  - No increasing risk of this cancer with increasing DMF exposure level or duration
  - All 11 cases reported heavy smoking for > 20 years

DuPont: Walrath *et al.* Case-Control Study

- Cancer cases from among over 8500 employees at 4 facilities
- 2 matched controls for each case
- 11 cases of testicular cancer
  - 8 cases at the 2 plants with lowest exposure levels to DMF (Plants A & D)
  - 3/11 cases and 6/22 controls exposed to DMF
  - Odds ratio = 1.00 (0.2, 5.1)

OEHHA Re-Analyses of DuPont Cohort Study

- **OEHHA claim that "statistics reported by Chen *et al.* (1988a) were calculated incorrectly"**
- **Chen *et al.* (1988a) used exact Poisson-based statistics for SIR estimates**
- **Chi-square test used in OEHHA reanalysis is wrong approach for SIR calculations with < 2 expected cases (Checkoway *et al.* 2004)**
- **Appropriate interpretation of 95% CI does not indicate a 'significant excess' for SIR estimates**

Select Statistical Tests for DuPont Incidence Study for Cohort Exposed Only To DMF (OEHHA Table A1)

Cancer	Obs	Exp	SIR (95%CI)	Poisson p-value		X^2	X^2 p-value
Buccal/ pharynx	9	1.6	5.6 (2.6,10.7)	<0.001		34.23	<0.001
Melanoma (wage)	5	2.1	2.4 (0.8,5.6)	0.12		4.01	0.05
Prostate (salary)	3	0.9	3.3 (0.7,9.7)	0.13		4.90	0.03
Stomach	3	0.8	3.8 (0.8, 21.0)	0.09		6.05	0.01

Conclusions from Epidemiologic Evidence

- Agree with overall conclusion by OEHHA that more definitive epidemiologic studies are needed
- Lack of confirmatory epidemiologic evidence since original Ducatman hypothesis
- "Although cases of testicular cancer among people exposed to DMF have been reported, these findings have not been corroborated in (limited) epidemiological studies, and it thus unlikely that DMF is carcinogenic to humans" (WHO, 2001)
- "There is inadequate evidence in humans for the carcinogenicity of DMF" (IARC, 1999)

Conclusions

The weight of the evidence clearly does not show that DMF causes invasive cancer in humans or in animals:

Human Data:

- **No evidence that DMF causes testicular tumors in humans**

Animal Studies:

- **Cancer observed only at doses exceeding the MTD does not "clearly show" that the test substance is a carcinogen**

DMF does not meet criteria for listing under Proposition 65

- **DMF has not clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer**