Office of Health and
Environmental Assessment
Washington DC 20460

EPA/600/8-82-004FA
May 1985
External Review Draft

EPA

Addendum to the Health Assessment Document for Dichloromethane (Methylene Chloride):

Review Draft

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Updated Carcinogen Assessment of Dichloromethane (Methylene Chloride)

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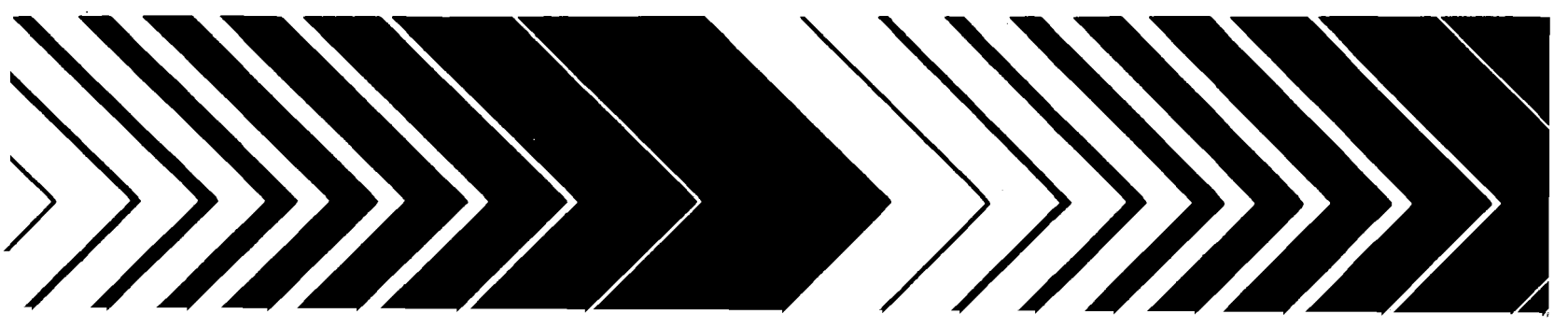

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Review Draft

ADDENDUM TO THE HEALTH ASSESSMENT DOCUMENT FOR DICHLOROMETHANE (METHYLENE CHLORIDE)

Updated Carcinogen Assessment of Dichloromethane (Methylene Chloride)

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Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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1. SUMMARY AND CONCLUSIONS

1.1. SUMMARY

1.1.1. Qualitative Assessment

There have been eight chronic studies in which dichloromethane (methylene chloride, DCM) was administered to animals: five in rats, two in mice, and one in hamsters. The Dow Chemical Company (1980) reported the results of chronic inhalation studies in rats and hamsters. There was a statistically significant increased incidence of ventral cervical sarcomas, probably of the salivary gland, consisting of sarcomas only, and appearing in males but not in females. In addition, the study showed a small increase in the number of benign mammary tumors compared to controls in female rats at all doses and in male rats at the highest dose. In hamsters, there was an increased incidence of lymphosarcoma in females which was not statistically significant after correction for survival. In a second inhalation study, the Dow Chemical Company (1982) reported that there was no increase in compound-related tumors in rats; however, the highest dose used in this study was far below that of the previous study. The National Coffee Association (1982a, b) conducted a study in which Fischer 344 rats and B6C3F1 mice were exposed to DCM in drinking water. The results indicated that Fischer 344 rats had an increased incidence of neoplastic nodules and/or hepatocellular carcinomas in female rats, which was significant with respect to matched controls; however, the incidence was within the range of historical control values at that laboratory. The National Coffee Association (1983) drinking water study in B6C3F1 mice also showed a borderline response of combined neoplastic nodules and hepatocellular carcinomas. The National Toxicology Program (1982) draft gavage study on rats and mice has not been published due to data discrepancies; however, usable infor-

mation from the gavage studies has been incorporated by the NTP into the inhalation bioassay (1985, draft).

The recently released NTP (1985) inhalation bioassay concluded that "there was some evidence of carcinogenicity of dichloromethane for male F344/N rats as shown by increased incidence of benign neoplasms of the mammary gland. There was clear evidence of carcinogenicity of dichloromethane for female F344/N rats as shown by increased incidence of benign neoplasms of the mammary gland. There was clear evidence of carcinogenicity of dichloromethane for male and female B6C3F1 mice, as shown by increased incidences of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms."

There are some other inadequate animal studies in the literature. One study (Theiss et al., 1977) reported a marginally positive pulmonary adenoma response in strain A mice injected intraperitoneally with DCM. Two negative animal inhalation studies were judged to be inadequate because they were not carried out for the full lifetime of the animals (Heppel et al., 1944; MacEwen et al., 1972).

Positive results in a rat embryo cell transformation study were reported by Price et al. (1978). The significance of their findings with regard to carcinogenicity is not well understood at the present time.

The epidemiologic data consist of two studies: Friedlander et al. (1978), updated by Hearne and Friedlander (1981), and Ott et al. (1983a, b, c, d, e). Although neither study showed excessive risk, both showed sufficient deficiencies to prevent them from being judged negative studies. The Friedlander et al. study (1978) lacked a large enough exposure (based on animal cancer potency estimates) to provide sufficient statistical power to detect a potential carcinogenic effect. The Ott et al. (1983a, b, c, d, e) study, among other deficiencies, lacked a sufficient latency period for site-specific cancer.

The NTP (1985) inhalation bioassay of DCM was conducted in male and female F344/N rats and B6C3F1 mice. The animals were exposed at concentrations of 0, 1000, 2000, and 4000 ppm for rats and 0, 2000, and 4000 ppm for mice, 6 hours/day, 5 days/week, for 102 weeks. There was an increased incidence of benign mammary gland neoplasms, primarily fibroadenomas, in both male and female rats. In female rats there was a significant increase in hepatocellular neoplastic nodules and hepatocellular carcinomas (combined) by the trend test only. There was also a statistically significant increase of mononuclear cell leukemias in female rats by age adjustment. In male rats there was a significant increase in mesotheliomas, primarily in the tunica vaginalis. Lastly, a marginally significant increase was noted in adrenal pheochromocytomas and interstitial cell tumors in male rats and pituitary gland adenomas and carcinomas combined, in male and female rats by the trend test only.

In the study using B6C3F1 mice, there was a highly significant increase in alveolar/bronchiolar adenoma and/or carcinoma in both sexes of mice. The incidence of hepatocellular adenoma and hepatocellular carcinoma combined was increased in the high-dose male group and in both dosed groups of female mice. It should be noted that there was also a dose-related increase in the number of mice bearing multiple lung and liver tumors. The control mice had no more than one lung tumor per mouse, whereas 38% of all dosed males and 42% of all dosed females had multiple lung tumors. The incidence of multiple hepatocellular tumors in the exposed groups increased in both sexes in a dose-related manner. Multiple hepatocellular tumors were found in only 4% of the male controls, and none were found in the female controls. In contrast, 28% of the males and 32% of the exposed females exhibited multiple liver tumors.

The NTP concluded that, under the conditions of this bioassay, there was

some evidence of the carcinogenicity of DCM for male F344/N rats as shown by an increased incidence of benign neoplasms of the mammary gland. There was sufficient or clear evidence of the carcinogenicity of DCM for female F344/N rats as shown by an increased incidence of benign neoplasms of the mammary gland. There was sufficient or clear evidence of carcinogenicity in male and female B6C3F1 mice as shown by increased incidences of lung and liver tumors.

1.1.2. Pharmacokinetics/Metabolism

The available data have been analyzed to determine if there are qualitative or quantitative metabolic differences or similarities between species which may alter the assumptions used in estimating the carcinogenic risk arising from exposure to DCM.

The results of both *in vitro* and *in vivo* studies indicate that DCM is metabolized via two pathways. One pathway yields carbon monoxide as an end product, and the other pathway yields carbon dioxide as an end product with formaldehyde and formic acid as metabolic intermediates. Each pathway involves formation of a metabolically active intermediate that is theoretically capable of irreversibly binding to cellular macromolecules. A comparative analysis of the capability of various tissues to metabolize DCM indicates that the liver is the primary site of metabolism, with some metabolism taking place in the lung and kidney. An analysis of the available *in vivo* data suggests that when rats or mice are exposed to high concentrations of DCM they exhale more carbon dioxide and excrete more formic acid than carbon monoxide. At exposure to low concentrations of DCM, both pathways appear to be utilized about equally. At the present time the implications of these observations in assessing the carcinogenic potency of DCM are unclear.

A comparative analysis of the data from *in vivo* studies in mice, rats, and humans indicates that all three species metabolize DCM to carbon monoxide.

Both mice and rats metabolize DCM to carbon dioxide. There are no human data on the metabolism of DCM to carbon dioxide. However, based on uptake data, some investigators have speculated that this pathway is functional in humans.

At present, the available data are insufficient for the purpose of estimating doses at which metabolism is saturated. The data indicate that, at low doses, little unmetabolized DCM is exhaled. At high doses there is a significant exhalation of DCM immediately post-exposure. The available data do suggest that at high doses more DCM is taken up into the body. Currently, the data are insufficient to determine the relationship between exposure concentration and uptake. Based on this analysis it is concluded that the available data do not offer useful parameters for modifying the assumptions used in the calculation of the carcinogenic unit risk of DCM.

1.1.3. Quantitative Estimation

In the previous carcinogenicity evaluation of DCM, a quantitative estimate for the upper-bound incremental unit risk was developed on the basis of salivary gland region tumors seen in an inhalation study with male rats (U.S. EPA, 1985).

The upper-bound estimate of incremental unit risk has been re-evaluated using the results of the NTP inhalation bioassay (NTP, 1985). The risk calculations presented here are based primarily on the NTP findings of carcinogenicity to the liver and lung in male and female mice. The elevated mammary tumor incidence in female rats and mammary and subcutaneous tumor incidence in male rats were also used for risk analysis. In mice, both separate and combined analyses were conducted for benign and malignant tumor types. Risk calculations were made for mice developing either the lung or liver tumors in order to indicate the total risk associated with tumors of these two organs. There were no adequate metabolic or pharmacokinetic data to support any modifications to

the experimentally applied doses. Thus, risk calculations were based on the experimentally applied doses (in ppm using the study dose schedule), with subsequent adjustment to estimate human equivalent doses and risks. The multistage dose-response model, as incorporated in the GLOBAL83 computer program (with the number of terms restricted to the number of experimental dose groups minus one), is the primary model utilized in the analysis. Both maximum likelihood estimates (MLE) and 95% upper confidence limit (UCL) values for risk are given.

The multistage model was found to provide an adequate fit to the experimental data for the tumor sites, tumor pathology types, sexes, and species groups examined. The highest estimate of risk was obtained from the UCL value for combined adenoma and carcinoma response in the lung and/or liver of female mice. To provide comparison with the basic multistage risk estimates, additional calculations were made with other risk estimation approaches and models using the data on mice having lung or liver tumors.

An analysis which excluded animals that died before the first tumors developed produced similar risk estimates (results were within 10% for female mice with lung and/or liver adenomas and carcinomas combined).

A time-to-tumor analysis using the multistage model, as formulated in the WEIBULL82 computer program, was also applied to the data to determine if the inclusion of a time term would influence risk estimates. The time-to-tumor estimates for the UCL of risk at low dose were generally in good agreement with the multistage model.

The probit and dichotomous Weibull models, in both background-independent and background-additive formulations (using the RISK81 computer program), were applied to the mouse data for comparison with the multistage model. For combined lung and liver tumors in female mice, the background-additive formula-

tions of both the probit and Weibull models are in good agreement with the multistage model. The background-independent formulations of the probit and Weibull models lead to much lower risk estimates.

The quantitative risk estimates developed from the NTP inhalation bioassay data were compared for consistency with findings in earlier long-term bioassays of DCM conducted by the Dow Chemical Company and the National Coffee Association. These studies provide some evidence of DCM-induced tumors consistent with the NTP findings. Multistage model UCL calculations using the results from these studies are comparable to, and in some cases exceed, estimates for respective tumor sites in the NTP study. In addition, the Dow inhalation study in rats showed an increase in tumors of the salivary gland region; the multistage UCL risk estimates for mammary tumors in the NTP female rats (the highest risk finding for the NTP rats) exceed the corresponding risk estimate based on the salivary tumors by a factor of three.

Equivalent human dose and upper-bound incremental unit risk estimates were developed using the standard assumptions of the Carcinogen Assessment Group on the inhalation rates of rodents and humans, and use of the body weight, to the two-thirds power interspecies extrapolation factor.

Using the multistage UCL estimates for female mice with either adenomas or carcinomas of the lung and/or liver, the upper-limit incremental unit risk from exposure over a lifetime to 1 mg/kg/day DCM is 1.4×10^{-2}. Equivalently, the unit risk for inhaling 1 $\mu g/m^3$ DCM over a lifetime is 4.1×10^{-6}; the unit risk for exposure to 1 ppm DCM is 1.4×10^{-2}.

Estimates of the incremental unit risk from exposure to DCM in drinking water were made using two approaches: first, based on the findings of liver, but not lung, tumors in the NTP inhalation bioassay with mice, and secondly, using the suggestively positive finding of liver tumors in the National Coffee

Association (1983) ingestion study in mice. Since the risk estimates from these two studies are roughly comparable, the mean of the derived risk values is chosen for the unit risk estimate via ingestion. Using the mean of the UCL risk calculations from these two studies, consumption of drinking water containing 1 μ g/L DCM over a lifetime has an associated upper-bound incremental unit risk estimate of 2.1×10^{-7}.

The upper-bound incremental unit risk for inhalation exposure estimated using the NTP bioassay was compared with the findings of the strongest epidemiologic study of workers exposed to DCM. While the epidemiologic study did not show any evidence for the carcinogenicity of DCM, power calculations showed that the study also did not have the power to detect the estimated increase with any degree of confidence.

1.2. CONCLUSIONS

Animal studies showed a statistically positive salivary gland sarcoma response in male rats (Dow Chemical Company, 1980) and a borderline hepatocellular neoplastic nodule response in female rats (National Coffee Association, 1982a, b). There is some evidence of the carcinogenicity of DCM in male rats, as shown by an increased incidence of benign mammary gland neoplasms; and clear evidence in female rats (Dow Chemical Company, 1980; Burek et al., 1984; NTP, 1985). There is clear evidence for the carcinogenicity of DCM in male and female mice, as shown by statistically significant increased incidences of alveolar/bronchiolar neoplasms and hepatocellular neoplasms (NTP, 1985 draft). There is also evidence that DCM is weakly mutagenic. Using the proposed EPA guidelines for carcinogen risk assessment (U.S. EPA, 1984), the weight-of-evidence ranking for the carcinogenicity of DCM in experimental animals is "sufficient," and for human evidence the ranking is "inadequate." Overall, an EPA category of B2 is assigned to DCM, meaning that DCM is to be

considered a "probable" human carcinogen. According to the criteria of the International Agency for Research on Cancer (IARC), the weight-of-evidence for the carcinogenicity of DCM in animals is "sufficient," placing it in Group 2B. The upper-bound incremental unit risk for the inhalation of air contaminated with DCM is 4.1×10^{-6} $(\mu g/m^3)^{-1}$ -[B2]. The upper-bound incremental unit risk for drinking water is 0.21×10^{-6} $(\mu g/L)^{-1}$ -[B2]. The CAG potency index for DCM is 1.2 $(mmol/kg/day)^{-1}$, which places DCM in the lowest quartile of the ranking of the chemicals that the CAG has evaluated as suspect carcinogens.

2. INTRODUCTION

In February of 1985 the Office of Health and Environmental Assessment published a Health Assessment Document on Dichloromethane (Methylene Chloride). The document, which contained an analysis of evidence for the carcinogenic potential of dichloromethane (DCM), concluded: "Using the criteria of the International Agency for Research on Cancer (IARC), the weight of evidence for carcinogenicity in animals is judged to be limited. . . . based upon the statistically positive salivary gland sarcoma response in male rats (Dow Chemical Company, 1980) and the borderline hepatocellular neoplastic nodule response in the rat and hepatocellular adenoma and/or carcinoma in male mice (National Coffee Association, 1982-1983)." It was further concluded: "When the absence of epidemiological evidence is considered along with the limited animal evidence, as well as the potential for DCM to cause gene mutations in mammalian systems, DCM is judged to be in IARC Group 3 . . . ." Because the National Toxicology Program (NTP) inhalation bioassay has been completed (NTP, 1985, draft) and the report has been reviewed and approved by the NTP Board of Scientific Councillors, it is necessary to update the February 1985 Health Assessment Document for Dichloromethane (Methylene Chloride).

The purpose of this addendum is to:

- Review and integrate the data obtained in the NTP inhalation bioassay,
- Analyze the pharmacokinetic/metabolic data presented in Chapter 4 of the Health Assessment Document and determine its usefulness in the quantitative estimation of carcinogenic risk, and
- Revise the estimated carcinogenic potency for DCM using the data from the NTP bioassay and pharmacokinetic data if appropriate.

3. CARCINOGENICITY

3.1 NATIONAL TOXICOLOGY PROGRAM INHALATION BIOASSAY (1985, DRAFT)

A 2-year carcinogenesis study of DCM (99% pure) was conducted at Battelle Pacific Northwest Laboratories by inhalation exposure to groups of 50 male and female F344/N rats and B6C3F1 mice (6 hours/day, 5 days/week) for 102 weeks. The exposure concentrations used were 0, 1000, 2000, or 4000 ppm for rats and 0, 2000, or 4000 ppm for mice. These doses were selected on the basis of results obtained from a 13-week subchronic inhalation study in which animals were exposed to concentrations of 525 to 8400 ppm 6 hours/day, 5 days/week. The maximum exposure concentration of 4000 ppm was selected because mimimal histopathologic changes were found after exposure to 4000 ppm. The second dose was 2000 ppm for both species. The third dose, 1000 ppm, was added for rats because in an earlier inhalation study in male and female Sprague-Dawley rats (Dow Chemical Company, 1980; Burek et al., 1984) reduced survival was observed in the highest exposure group, 3500 ppm.

All animals used in this experiment were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the carcinogenesis program of the National Toxicology Program (NTP). The rats were placed in the study at 7 to 8 weeks of age and mice at 8 to 9 weeks. All animals were housed individually. Food and water were available ad libitum except during exposure periods, when only water was available. All animals were observed twice a day for signs of moribundity or mortality. Clinical signs were recorded every week. Body weight was recorded once a week. A complete quality-controlled environment was maintained during the experiment. The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Tests of significance included pair-wise comparisons

of high-dose and low-dose groups with controls and tests for overall dose-response trends. Life table analysis, incidental tumor analysis, and the Fisher Exact Test were used to evaluate tumor incidence.

3.1.1. Rat Study

The mean body weights of experimental and control rats of each sex were similar throughout the studies (Figure 1). Rats exposed to 4000 ppm, the highest dose, were restless and pawed at the eyes and muzzle during the exposure period. The survival of male and female rats exposed to DCM is shown in Figure 2. The survival of female rats was significantly lower than that of the controls after week 100, and the survival in all groups of male rats at the termination of the study was low (Table 1). There were many deaths of males in the final 16 weeks of the study. The decreased survival is believed to be related to high incidence of leukemias.

There was a significant positive trend for mammary gland fibroadenoma and adenomas or fibroma (combined) in male and female rats. The incidence in high-dosed males (0/50, 0/50, 2/50, and 5/50) and in females (7/50, 13/50, 14/50, and 23/50) were significantly ($p < 0.001$) higher than the controls (Tables 2 and 3). Also, subcutaneous fibroma or sarcoma (combined), located in the mammary area in male rats, occurred with a significant positive trend ($p = 0.008$), and the incidence in the high-dose group was significantly ($p < 0.05$) greater than in the controls (Table 2). The subcutaneous tumors all occurred in the area of the mammary chain; therefore, the subcutaneous tumors were combined by the NTP for comparative purposes (male rats 1/50, 1/50, 4/50, and 9/50). The incidence of subcutaneous tumors in the highest dose group was significantly ($p = 0.002$) higher in than the controls. The historical incidence of mammary gland tumors at the same laboratory is 0% in males and 16% in females, and the NTP historical control incidence is 3% in males and 28% in females for the

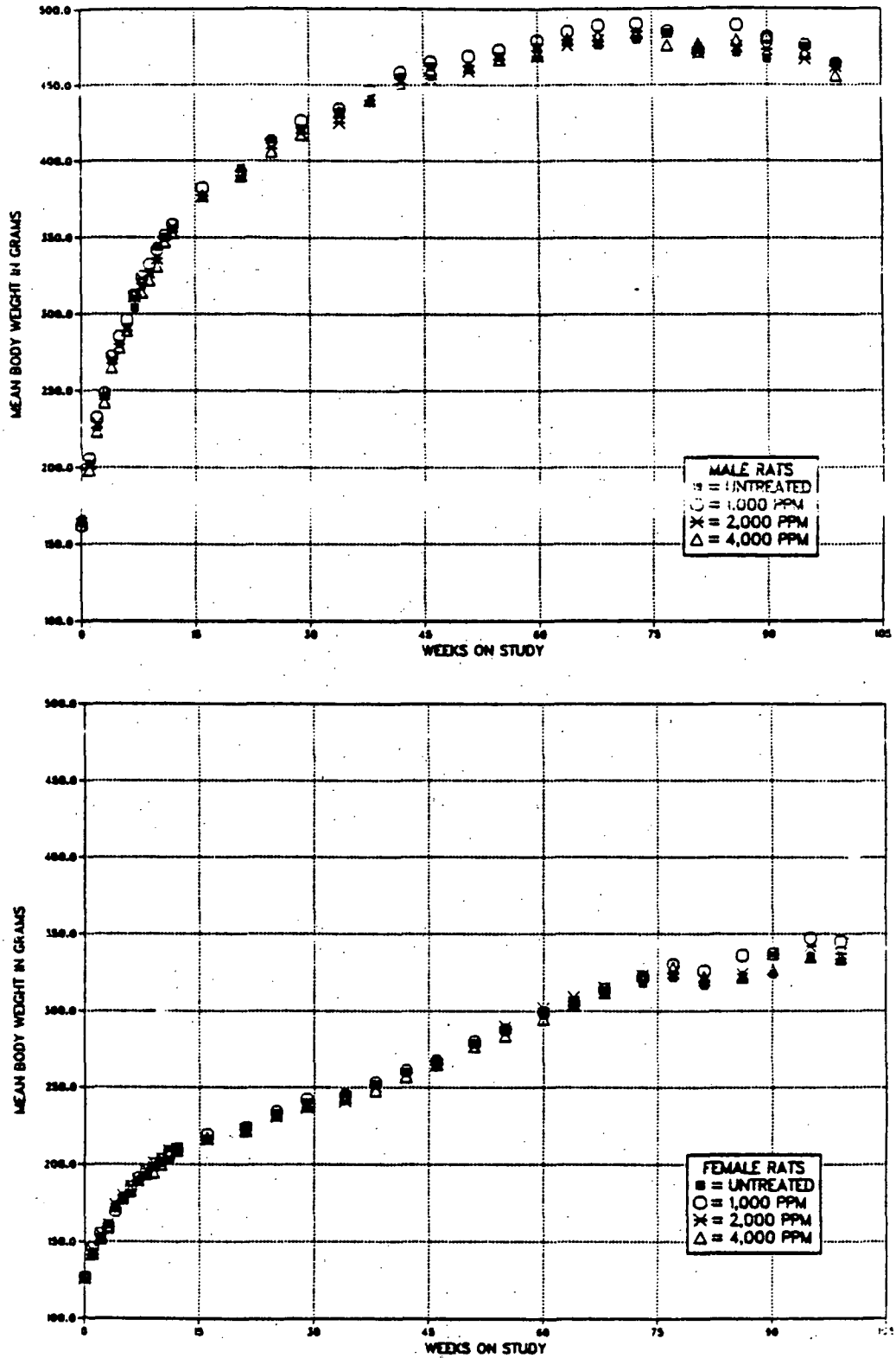

Figure 1. Growth curves for rats exposed to dichloromethane by inhalation for 2 years.

SOURCE: NTP, 1985.

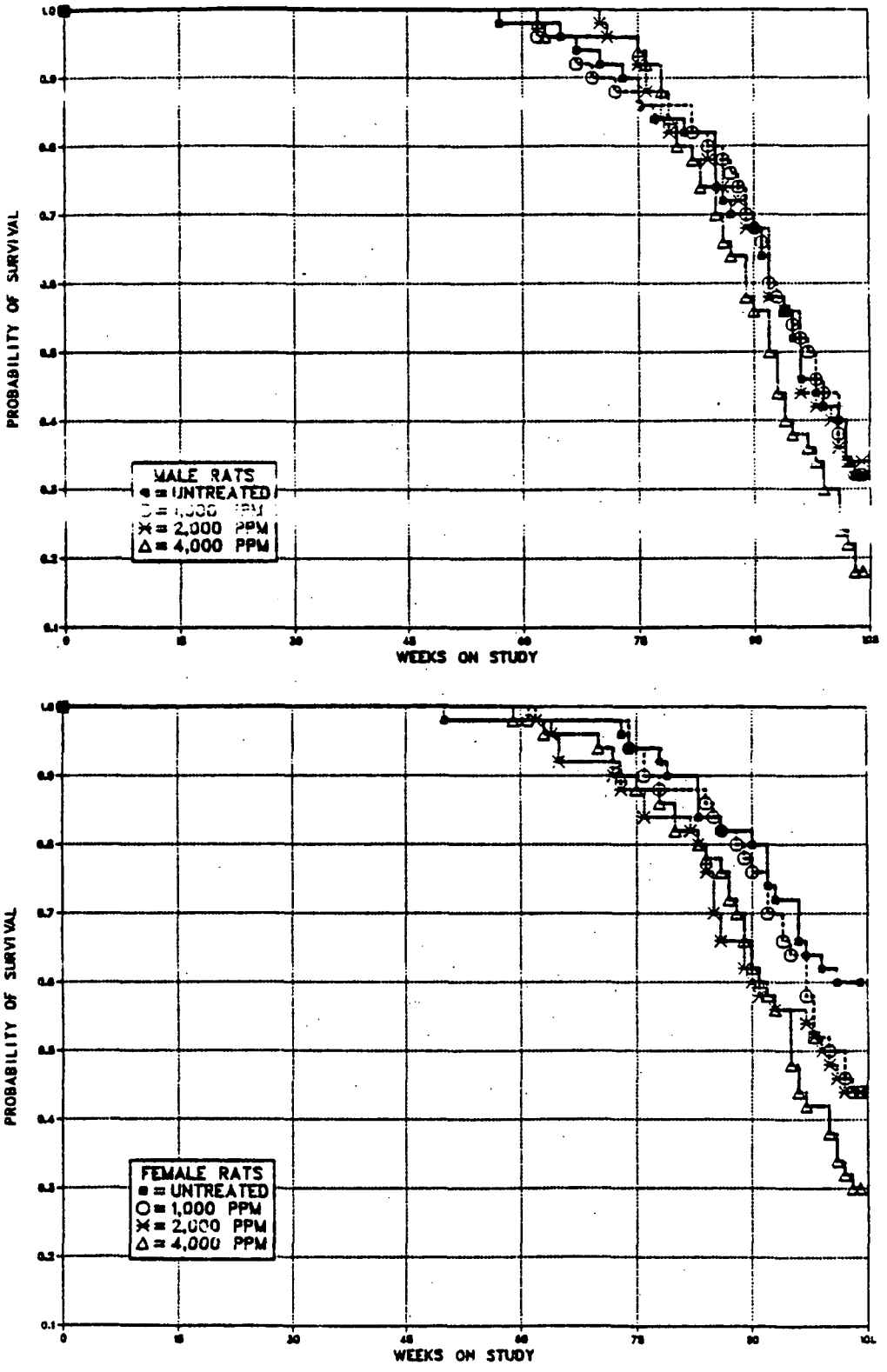

Figure 2. Kaplan-Meier survival curves for rats exposed to dichloromethane by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 1. SURVIVAL OF RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Male[a]				
Animals initially in study	50	50	50	50
Nonaccidental deaths before termination[b]	34	34	33	41
Killed at termination	16	16	17	9
Survival p values[c]	0.116	0.945	0.935	0.163
Female[a]				
Animals initially in study	50	50	50	50
Nonaccidental deaths before termination[b]	20	28	28	35
Killed at termination	30	22	22	15
Survival p values[c]	0.006	0.223	0.118	0.006

[a]Terminal kill period: week 104.
[b]Includes animals killed in a moribund condition.
[c]The results of the life table trend test are in the control column, and those of the life table pairwise comparisons with the controls are in the dosed columns.

SOURCE: NTP, 1985.

TABLE 2. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Subcutaneous tissue: Fibroma				
Overall rates[a]	1/50(2%)	1/50(2%)	2/50(4%)	4/50(8%)
Adjusted rates[b]	6.3%	6.3%	9.2%	19.5%
Terminal rates[c]	1/16(6%)	1/16(6%)	1/17(6%)	0/9(0%)
Week of first observation	104	104	96	89
Life table tests[d]	p=0.024	p=0.764	p=0.523	p=0.095
Incidental tumor tests[d]	p=0.064	p=0.764	p=0.505	p=0.204
Cochran-Armitage Trend Test[d]	p=0.072			
Fisher Exact Test[d]		p=0.753	p=0.500	p=0.181
Subcutaneous tissue: Fibroma or sarcoma				
Overall rates[a]	1/50(2%)	1/50(2%)	2/50(4%)	5/50(10%)
Adjusted rates[b]	6.3%	6.3%	9.2%	22.7%
Terminal rates[c]	1/16(6%)	1/16(6%)	1/17(6%)	0/9(0%)
Week of first observation	104	104	96	89
Life table tests[d]	p=0.008	p=0.764	p=0.523	p=0.050
Incidental tumor tests[d]	p=0.026	p=0.764	p=0.505	p=0.125
Cochran-Armitage Trend Test[d]	p=0.029			
Fisher Exact Test[d]		p=0.753	p=0.500	p=0.102
Hematopoietic system: Mononuclear cell leukemia				
Overall rates[a]	34/50(68%)	26/50(52%)	32/50(64%)	35/50(70%)
Adjusted rates[b]	80.3%	77.0%	80.2%	89.4%
Terminal rates[c]	8/16(50%)	9/16(56%)	10/17(59%)	6/9(67%)
Week of first observation	57	82	71	75
Life table tests[d]	p=0.045	p=0.147N	p=0.400N	p=0.134
Incidental tumor tests[d]	p=0.399	p=0.049N	p=0.434N	p=0.487N
Cochran-Armitage Trend Test[d]	p=0.251			
Fisher Exact Test[d]		p=0.076N	p=0.417N	p=0.500
Adrenal: Pheochromocytoma				
Overall rates[a]	5/50(10%)	11/50(22%)	10/50(20%)	10/50(20%)
Adjusted rates[b]	23.5%	46.4%	45.4%	52.9%
Terminal rates[c]	2/16(13%)	5/16(31%)	6/17(35%)	3/9(33%)
Week of first observation	75	89	89	80
Life table tests[d]	p=0.035	p=0.094	p=0.149	p=0.039
Incidental tumor tests[d]	p=0.131	p=0.093	p=0.131	p=0.108
Cochran-Armitage Trend Test[d]	p=0.192			
Fisher Exact Test[d]		p=0.086	p=0.131	p=0.131
Adrenal: Pheochromocytoma or pheochromocytoma, malignant				
Overall rates[a]	5/50(10%)	11/50(22%)	11/50(20%)	10/50(20%)
Adjusted rates[b]	23.5%	46.4%	47.0%	52.9%
Terminal rates[c]	2/16(13%)	5/16(31%)	6/17(35%)	3/9(33%)
Week of first observation	75	89	89	80
Life table tests[d]	p=0.034	p=0.094	p=0.104	p=0.039
Incidental tumor tests[d]	p=0.134	p=0.093	p=0.087	p=0.108
Cochran-Armitage Trend Test[d]	p=0.186			
Fisher Exact Test[d]		p=0.086	p=0.086	p=0.131

(continued on the following page)

TABLE 2. (continued)

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Mammary gland: Fibroadenoma				
Overall rates[a]	0/50(0%)	0/50(0%)	2/50(4%)	4/50(8%)
Adjusted rates[b]	0.0%	0.0%	11.8%	34.0%
Terminal rates[c]	0/16(0%)	0/16(0%)	2/17(12%)	2/9(22%)
Week of first observation			104	101
Life table tests[d]	p<0.001	e	p=0.250	p=0.020
Incidental tumor tests[d]	p<0.003	e	p=0.250	p=0.040
Cochran-Armitage Trend Test[d]	p=0.009			
Fisher Exact Test[d]		e	p=0.247	p=0.059
Mammary gland: Adenoma or fibroadenoma				
Overall rates[a]	0/50(0%)	0/50(0%)	2/50(4%)	5/50(10%)
Adjusted rates[b]	0.0%	0.0%	11.8%	36.6%
Terminal rates[c]	0/16(0%)	0/16(0%)	2/17(12%)	2/9(22%)
Week of first observation			104	93
Life table tests[d]	p<0.001	e	p=0.250	p=0.010
Incidental tumor tests[d]	p<0.001	e	p=0.250	p=0.023
Cochran-Armitage Trend Test[d]	p=0.003			
Fisher Exact Test[d]		e	p=0.247	p=0.028
Mammary gland or subcutaneous tissue: Adenoma, fibroadenoma, or fibroma				
Overall rates[a]	1/50(2%)	1/50(2%)	4/50(8%)	9/50(18%)
Adjusted rates[b]	6.3%	6.3%	20.6%	49.0%
Terminal rates[c]	1/16(6%)	1/16(6%)	3/17(18%)	2/9(22%)
Week of first observation	104	104	96	89
Life table tests[d]	p<0.001	p=0.764	p=0.196	p=0.002
Incidental tumor tests[d]	p=0.003	p=0.764	p=0.186	p=0.008
Cochran-Armitage Trend Test[d]	p<0.001			
Fisher Exact Test[d]		p=0.753N	p=0.181	p=0.008
Testis: Interstitial cell tumor				
Overall rates[a]	39/50(78%)	37/49(76%)	41/50(82%)	43/50(86%)
Adjusted rates[b]	94.9%	97.3%	95.2%	97.7%
Terminal rates[c]	14/16(88%)	15/16(94%)	15/17(88%)	8/9(89%)
Week of first observation	65	69	75	75
Life table tests[d]	p=0.009	p=0.420N	p=0.512	p=0.029
Incidental tumor tests[d]	p=0.114	p=0.385N	p=0.387	p=0.253
Cochran-Armitage Trend Test[d]	p=0.129			
Fisher Exact Test[d]		p=0.478N	p=0.401	p=0.218
Tunica vaginalis: Malignant mesothelioma				
Overall rates[a]	0/50(0%)	1/50(2%)	0/50(0%)	3/50(6%)
Adjusted rates[b]	0.0%	2.2%	0.0%	19.6%
Terminal rates[c]	0/16(0%)	0/16(0%)	0/17(0%)	0/9(0%)
Week of first observation		69		92
Life table tests[d]	p=0.025	p=0.496	e	p=0.068
Incidental tumor tests[d]	p=0.060	p=0.473	e	p=0.172
Cochran-Armitage Trend Test[d]	p=0.044			
Fisher Exact Test[d]		p=0.500	e	p=0.121

(continued on the following page)

TABLE 2. (continued)

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Tunica vaginalis: Mesothelioma (all types)				
Overall rates[a]	0/50(0%)	1/50(2%)	4/50(8%)	4/50(8%)
Adjusted rates[b]	0.0%	2.2%	19.2%	24.4%
Terminal rates[c]	0/16(0%)	0/16(0%)	2/17(12%)	0/9(0%)
Week of first observation		69	96	92
Life table tests[d]	p=0.009	p=0.496	p=0.070	p=0.031
Incidental tumor tests[d]	p=0.030	p=0.473	p=0.062	p=0.097
Cochran-Armitage Trend Test[d]	p=0.029			
Fisher Exact Test[d]		p=0.500	p=0.059	p=0.059
All sites: Malignant mesothelioma				
Overall rates[a]	0/50(0%)	2/50(4%)	0/50(0%)	3/50(6%)
Adjusted rates[b]	0.0%	4.4%	0.0%	19.6%
Terminal rates[c]	0/16(0%)	0/16(0%)	0/17(0%)	0/9(0%)
Week of first observation		69		92
Life table tests[d]	p=0.066	p=0.243	e	p=0.068
Incidental tumor tests[d]	p=0.136	p=0.225	e	p=0.172
Cochran-Armitage Trend Test[d]	p=0.097			
Fisher Exact Test[d]		p=0.247	e	p=0.121
All sites: Mesothelioma (all types)				
Overall rates[a]	0/50(0%)	2/50(4%)	5/50(10%)	4/50(8%)
Adjusted rates[b]	0.0%	4.4%	22.8%	24.4%
Terminal rates[c]	0/16(0%)	0/16(0%)	2/17(12%)	0/9(0%)
Week of first observation		69	96	92
Life table tests[d]	p=0.020	p=0.243	p=0.038	p=0.031
Incidental tumor tests[d]	p=0.063	p=0.225	p=0.030	p=0.097
Cochran-Armitage Trend Test[d]	p=0.052			
Fisher Exact Test[d]		p=0.247	p=0.028	p=0.059

[a]Number of tumor-bearing animals/number of animals examined at the site.
[b]Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality.
[c]Observed tumor incidence at terminal kill.
[d]Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparisons between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage Trend Test and the Fisher Exact Test directly compare the overall incidence rates. A negative trend or lower incidence in a dose group is indicated by the letter N.
[e]No p value is presented because no tumors were observed in the dosed and control groups.

Mammary gland fibroadenoma: Historical incidence at testing laboratory 0/100 (0%); historical incidence in NTP studies 51/1,727 (3%) ± 3%.
Mesothelioma--all sites: Historical incidence at testing laboratory 4/100 (4%); historical incidence in NTP studies 44/1,727 (3%) ± 2%.
Mononuclear cell leukemia: Historical incidence at testing laboratory 36/100 (36%); historical incidence in NTP studies 458/1,727 (27%) ± 9%.

SOURCE: NTP, 1985.

TABLE 3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Hematopoietic system: Mononuclear cell leukemia				
Overall rates[a]	17/50(34%)	17/50(34%)	23/50(46%)	23/50(46%)
Adjusted rates[b]	41.1%	44.4%	63.6%	58.1%
Terminal rates[c]	8/30(27%)	4/22(18%)	10/22(45%)	1/15(7%)
Week of first observation	73	76	73	63
Life table tests[d]	p=0.009	p=0.402	p=0.049	p=0.028
Incidental tumor tests[d]	p=0.273	p=0.425N	p=0.189	p=0.579
Cochran-Armitage Trend Test[d]	p=0.086			
Fisher Exact Test[d]		p=0.584N	p=0.154	p=0.154
Liver: Neoplastic nodule				
Overall rates[a]	2/50(4%)	1/50(2%)	3/50(6%)	5/50(10%)
Adjusted rates[b]	6.7%	2.4%	10.2%	19.6%
Terminal rates[c]	2/30(7%)	0/22(0%)	1/22(5%)	1/15(7%)
Week of first observation	104	61	85	73
Life table tests[d]	p=0.030	p=0.569N	p=0.382	p=0.080
Incidental tumor tests[d]	p=0.097	p=0.494N	p=0.482	p=0.229
Cochran-Armitage Trend Test[d]	p=0.078			
Fisher Exact Test[d]		p=0.500N	p=0.500	p=0.218
Liver: Neoplastic nodule or hepatocellular carcinoma				
Overall rates[a]	2/50(4%)	1/50(2%)	4/50(8%)	5/50(10%)
Adjusted rates[b]	6.7%	2.0%	14.4%	19.6%
Terminal rates[c]	2/30(7%)	0/22(0%)	2/22(9%)	1/15(7%)
Week of first observation	104	61	85	73
Life table tests[d]	p=0.027	p=0.569N	p=0.223	p=0.080
Incidental tumor tests[d]	p=0.086	p=0.494N	p=0.297	p=0.229
Cochran-Armitage Trend Test[d]	p=0.079			
Fisher Exact Test[d]		p=0.500N	p=0.339	p=0.218
Mammary gland: Fibroadenoma				
Overall rates[a]	5/50(10%)	11/50(22%)	13/50(26%)	22/50(44%)
Adjusted rates[b]	15.7%	41.2%	43.6%	79.4%
Terminal rates[c]	4/30(13%)	8/22(36%)	7/22(32%)	10/15(67%)
Week of first observation	96	74	65	73
Life table tests[d]	p<0.001	p=0.028	p=0.009	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.049	p=0.025	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001			
Fisher Exact Test[d]		p=0.086	p=0.033	p<0.001
Mammary gland: Adenoma or fibroadenoma				
Overall rates[a]	5/50(10%)	11/50(22%)	13/50(26%)	23/50(46%)
Adjusted rates[b]	15.7%	41.2%	43.6%	83.5%
Terminal rates[c]	4/30(13%)	8/22(36%)	7/22(32%)	11/15(73%)
Week of first observation	96	74	65	73
Life table tests[d]	p<0.001	p=0.028	p=0.009	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.049	p=0.025	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001			
Fisher Exact Test[d]		p=0.086	p=0.033	p<0.001

(continued on the following page)

TABLE 3. (continued)

	Control	1,000 ppm	2,000 ppm	4,000 ppm
Mammary gland: Adenoma, fibroadenoma, or adenocarcinoma				
Overall rates[a]	6/50(12%)	13/50(26%)	14/50(28%)	23/50(46%)
Adjusted rates[b]	17.8%	44.4%	44.9%	83.5%
Terminal rates[c]	4/30(13%)	8/22(36%)	7/22(32%)	11/15(73%)
Week of first observation	92	74	65	73
Life table tests[d]	p<0.001	p=0.023	p=0.012	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.053	p=0.043	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001			
Fisher Exact Test[d]		p=0.062	p=0.039	p<0.001
Mammary gland: Adenoma, fibroadenoma, adenocarcinoma, or mixed tumor, malignant				
Overall rates[a]	7/50(14%)	13/50(26%)	[e]14/50(28%)	23/50(46%)
Adjusted rates[b]	20.0%	44.4%	44.9%	83.5%
Terminal rates[c]	4/30(13%)	8/22(36%)	7/22(32%)	11/15(73%)
Week of first observation	92	74	65	73
Life table tests[d]	p<0.001	p=0.045	p=0.022	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.092	p=0.083	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001			
Fisher Exact Test[d]		p=0.105	p=0.070	p<0.001

[a]Number of tumor-bearing animals/number of animals examined at the site.
[b]Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality.
[c]Observed tumor incidence at terminal kill.
[d]Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparisons between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage Trend Test and the Fisher Exact Test directly compare the overall incidence rates. A negative trend or lower incidence in a dose group is indicated by the letter N.
[e]A carcinoma was also present in one of the animals that had a fibroadenoma.

Mammary gland fibroadenoma: Historical incidence at testing laboratory 16/99 (16%); historical incidence in NTP studies 492/1,772 (28%) ± 10%.
Mononuclear cell leukemia: Historical incidence at testing laboratory 27/99 (27%); historical incidence in NTP studies 307/1,772 (17%) ± 6.

SOURCE: NTP, 1985.

same strain of rats. The increased incidence of mammary gland tumors is consistent with the results reported by Dow Chemical Company (1980) and Burek et al. (1984) in Sprague-Dawley rats (U.S. EPA, 1985). This study has been reviewed previously. Sprague-Dawley rats have a spontaneous incidence of mammary gland tumors, about 80% in female and 10% in males. In males (Burek et al., 1984) the mammary tumors increased in the highest dose group to 14/97, as compared to 7/92 in the controls. In females the number of tumors per rat increased with dose. The increased incidence of benign mammary gland tumors in males and females provides some supportive evidence for mammary gland carcinogenesis. Maltoni (1984) also reported at the Food Solvent Workshop (March 8-9, 1984) on a study in which DCM was administered by gavage at 500 mg/kg/day, 5 days/week for 64 weeks, followed by an observation period until spontaneous death. Maltoni (1984) observed an increased incidence of mammary gland tumors in Sprague-Dawley rats. Thus, the incidence of mammary gland benign tumors in female rats in the present NTP study is consistent with the reports of Burek et al. (1984), Maltoni (1984), and Nitschke et al. (1982).

The incidence of liver neoplastic nodules or hepatocellular carcinomas (combined) in female rats (2/50, 1/50, 4/50, and 5/50) occurred with positive trends ($p = 0.030$) by life table analysis only (Table 3). The incidence in the high-dose group was not significantly greater than in the controls; this result is consistent with observations made by other investigators (Maltoni, 1984; NTP, 1982, unpublished; National Coffee Association, 1982a, b). Maltoni (1984) reported that gavage administration of 100 or 500 mg/kg/day for 64 weeks induced a dose-related increase in the incidence of nodular hyperplasia of the liver in Sprague-Dawley rats. The earlier gavage study (NTP, 1982, unpublished) indicated that administration of 500 or 1000 mg/kg/day increased the incidence of hepatocellular nodules in both male and female F344/N rats.

Furthermore, there was a significant increase in liver tumors in female rats dosed at 250 mg/kg/day in the drinking water study (National Coffee Association, 1982), although the number of tumors were within the range of the historical control values of the laboratory. The pharmacokinetic data presented by Dr. Kirshman at the Food Solvent Workshop (1984, page 41) indicated that 250 mg/kg/day in the drinking water study was equivalent to a 750 ppm inhalation level, which is 1/5 of the MTD used in the Burek et al. (1984) study and in the NTP (1985) study. The NTP reported that the highest exposure concentration (4000 ppm) in the inhalation study has been estimated to be equivalent to 1300 mg/kg/day from an oral dose.

In male rats the incidence of mesothelioma arising from tunica vaginalis (0/5, 2/50, 5/50, and 4/50) occurred with a significant positive trend ($p = 0.02$); the incidence in the mid- and high-dose groups was significantly higher ($p = 0.038$, $p = 0.30$) than in the controls (Table 2). This increased incidence may not be due to administration of DCM because the concurrent controls in the same laboratory were low in comparison with earlier inhalation studies (4/100). Mononuclear cell leukemia in male (Table 2) and female (Table 3) rats occurred with a significant positive trend by life table analysis only. The incidence (17/50, 17/50, 23/50, 23/50) in females was significantly greater than in the controls at the mid-dose ($p = 0.049$) and high-dose ($p = 0.028$) levels.

Some other tumor incidences were increased marginally in experimental groups as compared to the controls. These increases were characterized by a significant trend only. These tumors included adrenal gland pheochromocytoma and interstitial cell tumors in males (Table 2) and pituitary gland adenoma or carcinoma (combined) in males and females (Tables 2 and 3). The squamous cell metaplasia of the nasal cavity in female rats (1/50, 2/50, 3/50, and 9/50) was increased significantly in the high-dose group, but no nasal tumors were

found in this group.

3.1.2. Mouse Study

The mean initial body weight of males in the 4000 ppm group was 15% lower than that of controls (Figure 3). The mean body weights were comparable in the high-dose and control groups until week 90, but after week 90 the body weights were 8% to 11% lower than those of controls. During the exposure period, the mice were hyperactive. The probabilities of survival of male and female mice are shown in Figure 4. The survival in both male and female high-dose groups decreased significantly compared with controls (Table 4). The reduced survival may have been due to chemically-induced lung and liver tumors in both male and female mice.

The incidences of lung tumors increased significantly ($p = 0.0001$) in both males (Table 5) and females (Table 6). The latent period for tumor induction was significantly (trend analysis) decreased in the high-dose groups as compared to controls. The tumors observed were alveolar/bronchiolar adenomas (males, 3/50, 19/50, and 24/50; females, 2/50, 23/48, and 28/48) and alveolar/bronchiolar carcinomas (males, 2/50, 10/50, and 28/50; females, 1/50, 13/48, 29/48, and 29/48). In addition to the dose-related increase in lung tumors in male and female mice, there were dose-related increases in multiple lung tumor-bearing mice (Table 7). The multiplicity of tumors included both alveolar/bronchiolar adenoma and carcinoma. Only one lung tumor per mouse was found in the controls, whereas 70% of the high-dose males and females had multiple tumors (males, 0/50, 10/50, and 28/50; females, 0/50, 11/48, and 29/48). In the experimental groups, 38% of the dosed male mice and 42% of the dosed female mice had multiple lung tumors. These results are consistent with the data obtained in other studies. In the earlier NTP (1982, unpublished) gavage study, DCM produced a significant increase in lung tumors in female mice. Maltoni (1984) also

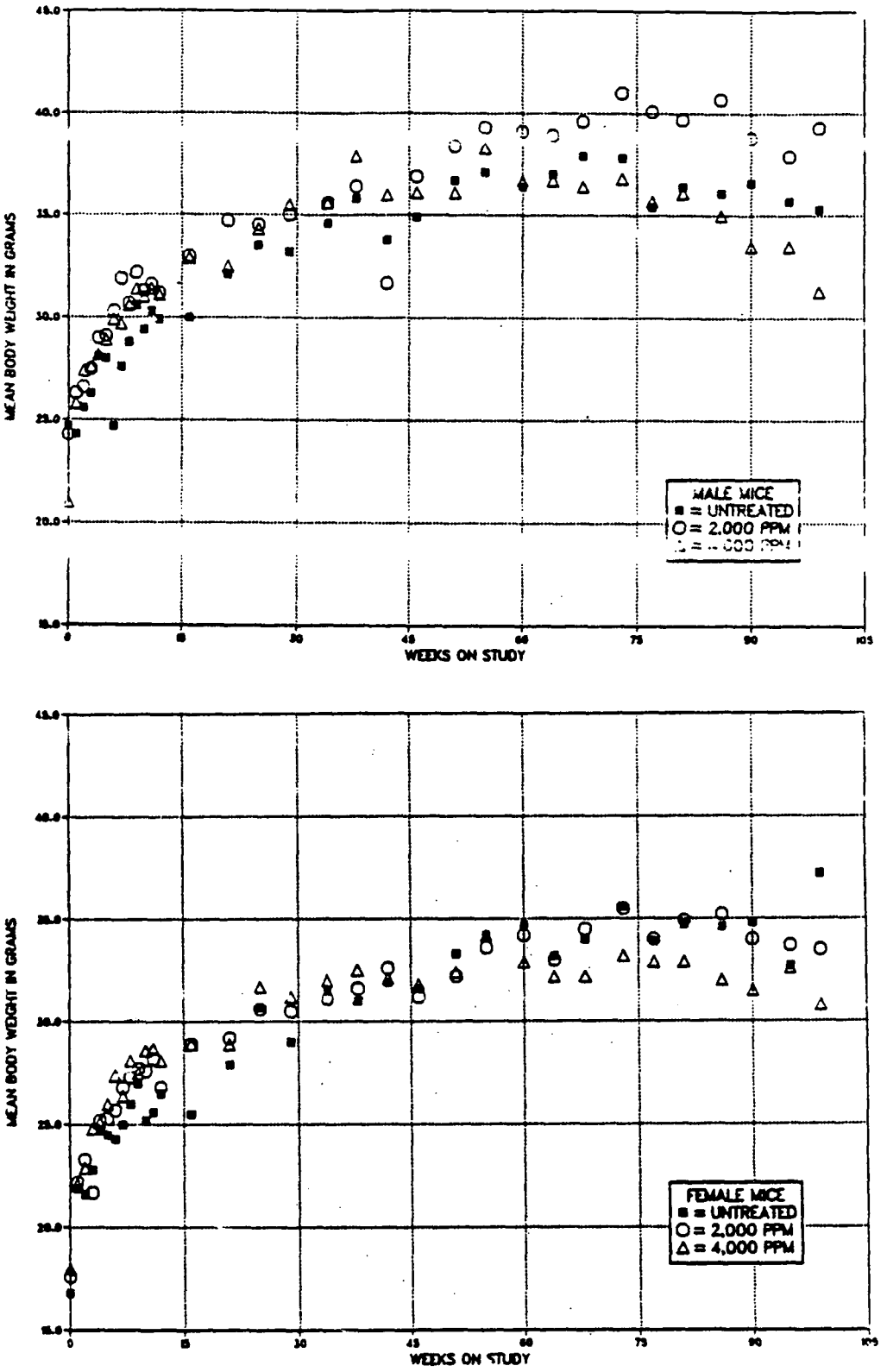

Figure 3. Growth curves for mice exposed to dichloromethane by inhalation for 2 years.

SOURCE: NTP, 1985.

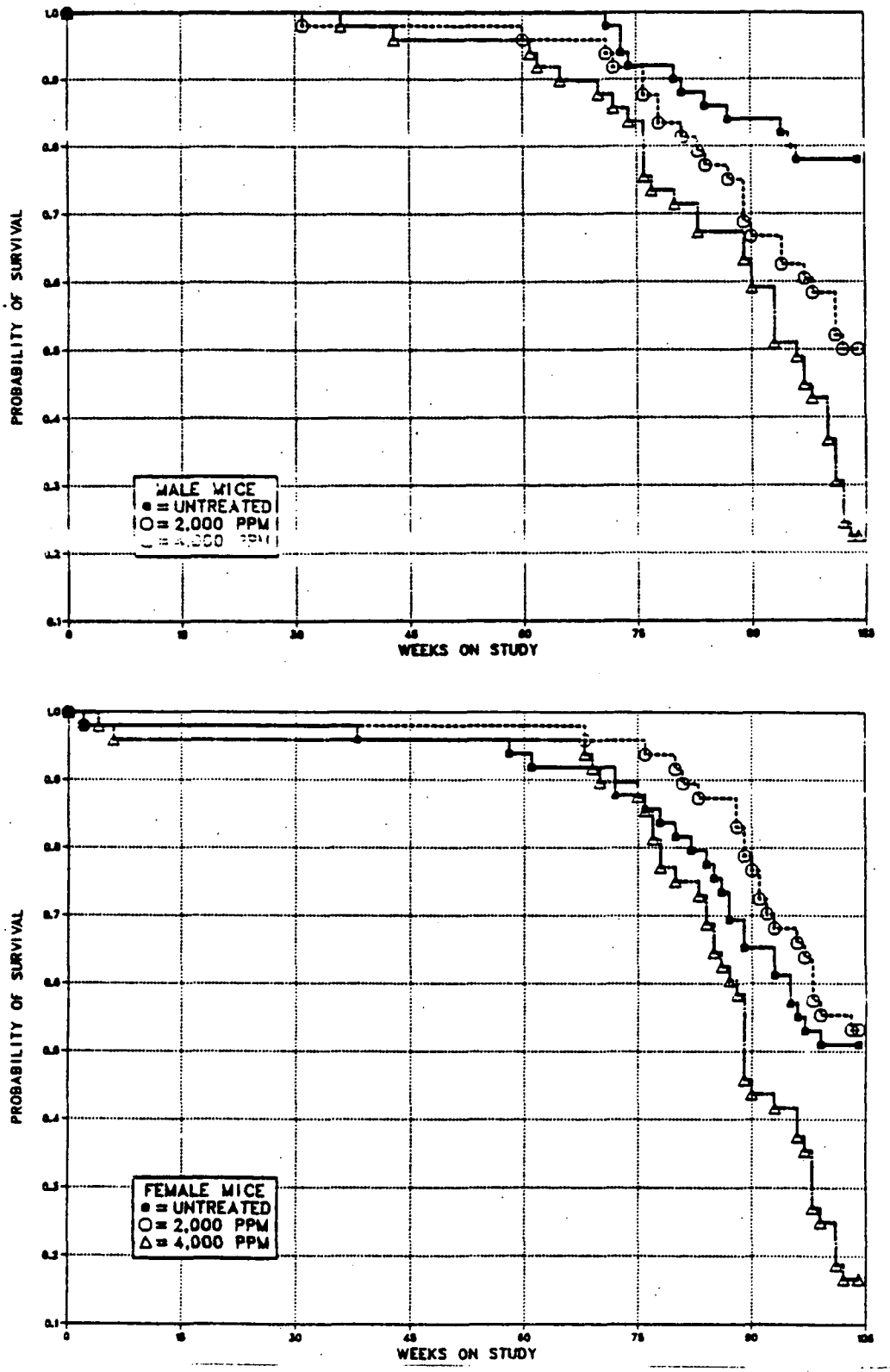

Figure 4. Kaplan-Meier survival curves for mice exposed to dichloromethane by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 4. SURVIVAL OF MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	2,000 ppm	4,000 ppm
Male[a]			
Animals initially in study	50	50	50
Nonaccidental deaths before termination[b]	11	24	38
Accidentally killed	0	2	1
Killed at termination	39	24	9
Died during termination period	0	0	2
Survival p values[c]	<0.001	<0.010	<0.001
Female[a]			
Animals initially in study	50	50	50
Nonaccidental deaths before termination[b]	24	22	40
Accidentally killed	1	2	1
Killed at termination	0	1	1
Died during termination period	25	25	8
Survival p values[c]	0.002	0.678	0.004

[a]Terminal kill period: week 104.
[b]Includes animals killed in a moribund condition.
[c]The results of the life table trend test are in the control column, and those of the life table pairwise comparisons with the controls are in the dosed columns.

SOURCE: NTP, 1985.

TABLE 5. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	2,000 ppm	4,000 ppm
Lung: Alveolar/bronchiolar adenoma			
Overall rates[a]	3/50(6%)	19/50(38%)	24/50(48%)
Adjusted rates[b]	7.7%	55.6%	78.5%
Terminal rates[c]	3/39(8%)	10/24(42%)	6/11(55%)
Week of first observation	104	71	70
Life table tests[d]	p<0.001	p<0.001	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.001	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Lung: Alveolar/bronchiolar carcinoma			
Overall rates[a]	2/50(4%)	10/50(20%)	28/50(56%)
Adjusted rates[b]	4.9%	34.0%	92.9%
Terminal rates[c]	1/39(3%)	6/24(25%)	9/11(82%)
Week of first observation	94	78	72
Life table tests[d]	p<0.001	p<0.002	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.016	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.014	p<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma			
Overall rates[a]	5/50(10%)	27/50(54%)	40/50(80%)
Adjusted rates[b]	12.4%	74.2%	100.0%
Terminal rates[c]	4/39(10%)	15/24(63%)	11/11(100%)
Week of first observation	94	71	70
Life table tests[d]	p<0.001	p<0.001	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.001	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Circulatory system: Hemangiosarcoma			
Overall rates[a]	1/50(2%)	2/50(4%)	5/50(10%)
Adjusted rates[b]	2.6%	7.6%	21.4%
Terminal rates[c]	1/39(3%)	1/24(4%)	1/11(9%)
Week of first observation	104	101	70
Life table tests[d]	p=0.007	p=0.352	p=0.017
Incidental tumor tests[d]	p=0.083	p=0.495	p=0.142
Cochran-Armitage Trend Test[d]	p=0.060		
Fisher Exact Test[d]		p=0.500	p=0.102
Circulatory system: Hemangioma or hemangiosarcoma			
Overall rates[a]	2/50(4%)	2/50(4%)	6/50(12%)
Adjusted rates[b]	4.8%	7.6%	25.8%
Terminal rates[c]	1/39(3%)	1/24(4%)	1/11(9%)
Week of first observation	87	101	70
Life table tests[d]	p=0.010	p=0.558	p=0.022
Incidental tumor tests[d]	p=0.170	p=0.643N	p=0.301
Cochran-Armitage Trend Test[d]	p=0.080		
Fisher Exact Test[d]		p=0.691	p=0.134

(continued on the following page)

TABLE 5. (continued)

	Control	2,000 ppm	4,000 ppm
Liver: Hepatocellular adenoma			
Overall rates[a]	10/50(20%)	14/49(29%)	14/49(29%)
Adjusted rates[b]	23.0%	46.9%	68.3%
Terminal rates[c]	7/39(18%)	9/24(38%)	6/11(55%)
Week of first observation	73	71	80
Life table tests[d]	p<0.001	p=0.041	p=0.001
Incidental tumor tests[d]	p<0.075	p=0.161	p=0.095
Cochran-Armitage Trend Test[d]	p=0.194		
Fisher Exact Test[d]		p=0.224	p=0.224
Liver: Hepatocellular carcinoma			
Overall rates[a]	13/50(26%)	15/49(31%)	26/49(53%)
Adjusted rates[b]	29.7%	43.7%	76.4%
Terminal rates[c]	9/39(23%)	7/24(29%)	5/11(45%)
Week of first observation	73	72	61
Life table tests[d]	p<0.001	p=0.111	p<0.001
Incidental tumor tests[d]	p=0.016	p=0.422	p=0.042
Cochran-Armitage Trend Test[d]	p=0.004		
Fisher Exact Test[d]		p=0.387	p=0.005
Liver: Hepatocellular adenoma or carcinoma			
Overall rates[a]	22/50(44%)	22/49(49%)	33/49(67%)
Adjusted rates[b]	48.3%	66.8%	93.0%
Terminal rates[c]	16/39(41%)	13/24(54%)	9/11(82%)
Week of first observation	73	71	61
Life table tests[d]	p<0.001	p=0.048	p<0.001
Incidental tumor tests[d]	p=0.010	p=0.305	p=0.020
Cochran-Armitage Trend Test[d]	p=0.013		
Fisher Exact Test[d]		p=0.384	p=0.016

[a]Number of tumor-bearing animals/number of animals examined at the site.
[b]Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality.
[c]Observed tumor incidence at terminal kill.
[d]Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparisons between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage Trend Test and the Fisher Exact Test directly compare the overall incidence rates. A negative trend or lower incidence in a dose group is indicated by the letter N.

Alveolar/bronchiolar adenoma or carcinoma: Historical incidence at testing laboratory 31/100 (31%); historical incidence in NTP studies 296/1,780 (17%) ± 8%.
Hepatocellular adenoma or carcinoma: Historical incidence at testing laboratory 28/100 (28%); historical incidence in NTP studies 540/1,784 (30%) ± 8%.
Hemangioma or hemangiosarcoma: Historical incidence at testing laboratory 2/100 (2%); historical incidence in NTP studies 78/1,791 (4%) ± 4%.

SOURCE: NTP, 1985.

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Control	2,000 ppm	4,000 ppm
Lung: Alveolar/bronchiolar adenoma			
Overall rates[a]	2/50(4%)	23/48(48%)	28/48(58%)
Adjusted rates[b]	6.7%	66.5%	91.1%
Terminal rates[c]	1/25(4%)	14/25(56%)	6/8(75%)
Week of first observation	87	83	68
Life table tests[d]	p<0.001	p<0.001	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.001	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Lung: Alveolar/bronchiolar carcinoma			
Overall rates[a]	1/50(4%)	13/48(27%)	29/48(60%)
Adjusted rates[b]	4.0%	45.9%	92.2%
Terminal rates[c]	1/25(4%)	10/25(40%)	6/8(75%)
Week of first observation	104	89	68
Life table tests[d]	p<0.001	p<0.001	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.001	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Lung: Alveolar/bronchiolar adenoma or carcinoma			
Overall rates[a]	3/50(6%)	30/48(63%)	41/48(85%)
Adjusted rates[b]	10.6%	82.9%	100.0%
Terminal rates[c]	2/25(8%)	19/25(76%)	8/8(100%)
Week of first observation	87	83	68
Life table tests[d]	p<0.001	p<0.001	p<0.001
Incidental tumor tests[d]	p<0.001	p<0.001	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Liver: Hepatocellular adenoma			
Overall rates[a]	2/50(4%)	6/48(13%)	22/48(46%)
Adjusted rates[b]	6.5%	21.3%	83.0%
Terminal rates[c]	1/25(4%)	4/25(16%)	5/8(63%)
Week of first observation	84	96	68
Life table tests[d]	p<0.001	p=0.151	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.155	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p=0.121	p<0.001
Liver: Hepatocellular carcinoma			
Overall rates[a]	1/50(2%)	11/48(23%)	32/48(67%)
Adjusted rates[b]	4.0%	34.0%	96.5%
Terminal rates[c]	1/25(4%)	6/25(24%)	7/8(88%)
Week of first observation	104	83	68
Life table tests[d]	p<0.001	p=0.005	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.004	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p=0.001	p<0.001

(continued on the following page)

TABLE 6. (continued)

	Control	2,000 ppm	4,000 ppm
Liver: Hepatocellular adenoma or carcinoma			
Overall rates[a]	3/50(6%)	16/48(33%)	40/48(83%)
Adjusted rates[b]	10.4%	48.0%	100.0%
Terminal rates[c]	2/25(8%)	9/25(36%)	8/8(100%)
Week of first observation	84	83	68
Life table tests[d]	p<0.001	p=0.002	p<0.001
Incidental tumor tests[d]	p<0.001	p=0.002	p<0.001
Cochran-Armitage Trend Test[d]	p<0.001		
Fisher Exact Test[d]		p<0.001	p<0.001
Thyroid gland: Follicular cell adenoma			
Overall rates[a]	1/48(2%)	1/47(2%)	4/46(9%)
Adjusted rates[b]	4.2%	4.0%	35.0%
Terminal rates[c]	1/24(4%)	1/25(4%)	2/8(25%)
Week of first observation	104	104	77
Life table tests[d]	p=0.012	p=0.754N	p=0.022
Incidental tumor tests[d]	p=0.040	p=0.754N	p=0.069
Cochran-Armitage Trend Test[d]	p=0.093		
Fisher Exact Test[d]		p=0.747	p=0.168

[a]Number of tumor-bearing animals/number of animals examined at the site.
[b]Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality.
[c]Observed tumor incidence at terminal kill.
[d]Beneath the control incidence are the p values associated with the trend test. Beneath the dosed group incidence are the p values corresponding to pairwise comparisons between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage Trend Test and the Fisher Exact Test directly compare the overall incidence rates. A negative trend or lower incidence in a dose group is indicated by the letter N.

Alveolar/bronchiolar adenoma or carcinoma: Historical incidence at testing laboratory 10/100 (10%); historical incidence in NTP studies 122/1,777 (7%) ± 4%.
Hepatocellular adenoma or carcinoma: Historical incidence at testing laboratory 5/100 (5%); historical incidence in NTP studies 147/1,781 (8%) ± 5%.

SOURCE: NTP, 1985.

TABLE 7. MULTIPLICITY OF PULMONARY TUMORS IN MICE EXPOSED TO DICHLOROMETHANE

Diagnoses	Exposure groups (ppm) 0	2,000	4,000
Male			
One adenoma and one carcinoma	0/50	1/50	3/50
Multiple adenomas	0/50	5/50	4/50
Multiple carcinomas	0/50	3/50	12/50
Multiple adenomas and multiple carcinomas	0/50	0/50	3/50
One adenoma and multiple carcinomas	0/50	1/50	3/50
Multiple adenomas and one carcinoma	0/50	0/50	3/50
Incidence of mice with multiple tumors	0/50(0%)	10/50(20%)	28/50(56%)
No. of mice with multiple tumors/no. of mice with pulmonary tumors	0/5(0%)	10/27(37%)	28/40(70%)
Female			
One adenoma and one carcinoma	0/50	2/48	4/48
Multiple adenomas	0/50	4/48	5/48
Multiple carcinomas	0/50	1/48	8/48
Multiple adenomas and multiple carcinomas	0/50	0/48	2/48
One adenoma and multiple carcinomas	0/50	2/48	7/48
Multiple adenomas and one carcinoma	0/50	2/48	3/48
Incidence of mice with multiple tumors	0/50(0%)	11/48(23%)	29/48(60%)
No. of mice with multiple tumors/no. of mice with pulmonary tumors	0/3(0%)	11/30(37%)	29/41(71%)

SOURCE: NTP, 1985.

reported an increased incidence of lung tumors (one and one-half times) in male mice dosed with 100 to 500 mg/kg/day DCM by gavage.

In male mice (Table 5) the hepatocellular adenoma or carcinoma (combined) (22/50, 24/49, and 33/49) and hepatocellular carcinoma (13/50, 15/49, and 26/49) were increased significantly ($p < 0.001$), especially at 4000 ppm. In female mice (Table 6), DCM produced dose-related increases in both hepatocellular adenoma (2/50, 6/48, and 22/48) and hepatocellular carcinoma (1/50, 11/48, and 32/48), which were highly significant by any statistical test. The incidence of these tumors in the controls was consistent with the historical control values of this laboratory. The multiplicity of the hepatocellular neoplasms was common in the male and female dosed mice (Table 8). It should be noted that only 4% of the male control mice and none of the female control mice had multiplicity of liver tumors. The multiplicity of hepatocellular tumors in both male and female mice increased significantly in a dose-related manner (males, 2/50, 11/49, and 16/46; females, 0/50, 3/48, and 28/48). There were 27/57 (47%) males and 31/56 (55%) females with multiple tumors. The increased incidence of hepatocellular tumors is consistent with the results of the previous NTP (1982, unpublished) gavage study, the Maltoni (1984) study, and the National Coffee Association (1983) drinking water study. The National Coffee Association study produced a borderline significant increase in liver tumors at a dose significantly less than the maximum tolerated dose. There was also an increase in hemangiosarcoma (1/50, 2/50, and 5/50) or hemangioma and hemangiosarcoma (2/50, 2/50, and 6/50) in male mice (Table 7), which occurred with a positive trend by life table analysis.

3.1.3. Summary

The results of the NTP inhalation bioassay (1985, draft) using F344/N rats showed an increased incidence of benign mammary gland neoplasms, primarily

TABLE 8. MULTIPLICITY OF LIVER TUMORS IN MICE EXPOSED TO DICHLOROMETHANE

Diagnoses	Exposure groups (ppm) 0	2,000	4,000
Male			
One adenoma and one carcinoma	1/50	2/49	3/49
Multiple adenomas	0/50	3/49	3/49
Multiple carcinomas	1/50	3/49	6/49
Multiple adenomas and multiple carcinomas	0/50	0/49	1/49
One adenoma and multiple carcinomas	0/50	0/49	2/49
Multiple adenomas and one carcinoma	0/50	3/49	1/49
Incidence of mice with multiple tumors	2/50(4%)	11/49(22%)	16/49(33%)
No. of mice with multiple tumors/no. of mice with pulmonary tumors	2/22(9%)	11/24(46%)	16/33(48%)
Female			
One adenoma and one carcinoma	0/50	1/48	6/48
Multiple adenomas	0/50	0/48	4/48
Multiple carcinomas	0/50	2/48	10/48
Multiple adenomas and multiple carcinomas	0/50	0/48	3/48
One adenoma and multiple carcinomas	0/50	0/48	1/48
Multiple adenomas and one carcinoma	0/50	0/48	4/48
Incidence of mice with multiple tumors	0/5(0%)	3/48(6%)	28/48(58%)
No. of mice with multiple tumors/no. of mice with pulmonary tumors	0/3(0%)	3/16(19%)	28/40(70%)

SOURCE: NTP, 1985.

fibroadenomas, in both male and female rats. In female rats, there was also a significant increase in hepatocellular neoplastic nodules and hepatocellular carcinomas (combined) by the trend test only and a statistically significant increase of mononuclear cell leukemias by age adjustment. In male rats, there was a significant increase in mesotheliomas, primarily from the tunica vaginalis. Lastly, there was a marginally significant increase in adrenal pheochromocytomas and interstitial cell tumors in males and pituitary gland adenomas and carcinomas (combined) in male and female rats by the trend test only.

In the NTP inhalation bioassay using B6C3F1 mice, a highly significant increase in alveolar/bronchiolar adenoma and/or carcinoma was observed in both sexes. The incidence of hepatocellular adenoma and hepatocellular carcinoma (combined) was increased in the high-dose males and in both dosed groups of females. There was also a dose-related increase in the number of mice bearing multiple lung and liver tumors. Only one lung tumor per mouse was found, whereas 38% of all dosed male mice and 42% of all dosed female mice had multiple lung tumors. The incidence of multiple hepatocellular tumors in the exposed groups increased in both sexes in a dose-related manner. Multiple hepatocellular tumors were found in only 4% of the male controls, but none were found in the female controls; in contrast, 28% of the exposed males and 32% of the exposed females exhibited multiple liver tumors.

The NTP concluded that, under the conditions of this bioassay, there was: some evidence of DCM carcinogenicity for male F344/N rats as shown by an increased incidence of benign neoplasms of the mammary gland, sufficient or clear evidence of DCM carcinogenicity for female F344/N rats as shown by an increased incidence of benign neoplasms of the mammary gland, and sufficient or clear evidence of DCM carcinogenicity in male and female B6C3F1 mice as shown by increased incidences of lung and liver tumors.

3.2. PHARMACOKINETICS/METABOLISM

The purpose of this analysis is to determine if there are available pharmacokinetic/metabolism data that can be useful in the assessment of the carcinogenic risk arising from exposure to methylene chloride (DCM). Most of the data used in this analysis have previously been reviewed (U.S. EPA, 1985) and therefore will not be discussed in detail. Data on routes of exposure, rates of ingestion, metabolism, and administered versus effective dose in the NTP bioassay have been reviewed in order to determine if there are qualitative or quantitative differences or similarities between species which may alter the assumptions used in estimating risks. Relevant new data have been incorporated as appropriate.

3.2.1. In Vitro Metabolism/Pathways

The preponderance of data obtained from both *in vivo* and *in vitro* experiments indicate that DCM and other dihalomethanes are biotransformed to both carbon monoxide and carbon dioxide. Carbon monoxide is the end product of microsomal oxidation, and carbon dioxide is an end product of cytosolic metabolism.

A number of investigators (Kubic and Anders, 1975, 1978; Hogan et al., 1976; Stevens and Anders, 1978, 1979; and Ahmed and Anders, 1978) have studied the metabolism of DCM and other dihalomethanes in experiments using rat liver microsomes. These studies have resulted in the following observations:

- NADPH and molecular oxygen are required for maximal activity;
- Anaerobic conditions reduce the rate of DCM conversion to carbon monoxide by 80 percent;
- There is a high correlation between the *in vitro* production of carbon monoxide and microsomal P_{450} content;
- Pretreatment of test animals with P_{450} inducers resulted in

increased conversion of DCM to carbon monoxide by rat liver microsomes;

- Pretreatment of test animals with P_{450} inhibitors resulted in decreased conversion of DCM to carbon monoxide by rat liver microsomes; and
- Cleavage of the carbon-hydrogen bond is the rate-limiting step in dihalomethane metabolism.

Based on these observations, Anders et al. (1977) postulated that DCM is biotransformed to carbon monoxide by the microsomal mixed-function oxidases via formation of a formyl halide intermediate. The proposed mechanism for the metabolism of dihalomethanes by liver microsomes is summarized in Figure 5.

A number of investigators (Heppel and Porterfield, 1948; Kubic and Anders, 1975; Ahmed and Anders, 1976, 1978) have studied the metabolism of DCM and other dihalomethanes to carbon dioxide, formaldehyde, and formic acid using a rat liver cytosolic fraction. They have made the following observations:

- The reaction does not require molecular oxygen;
- The reaction is glutathione-dependent;
- Chemicals known to complex with glutathione inhibit the reaction; and
- Removal of formaldehyde dehydrogenase by ammonium hydroxide fractionation results in the formation of formaldehyde only, and not formic acid.

Based on these observations, Ahmed et al. (1980) postulated that DCM is biotransformed to carbon dioxide by the liver cytosolic fraction via formation of a reactive formyl intermediate. The proposed mechanism for the metabolism

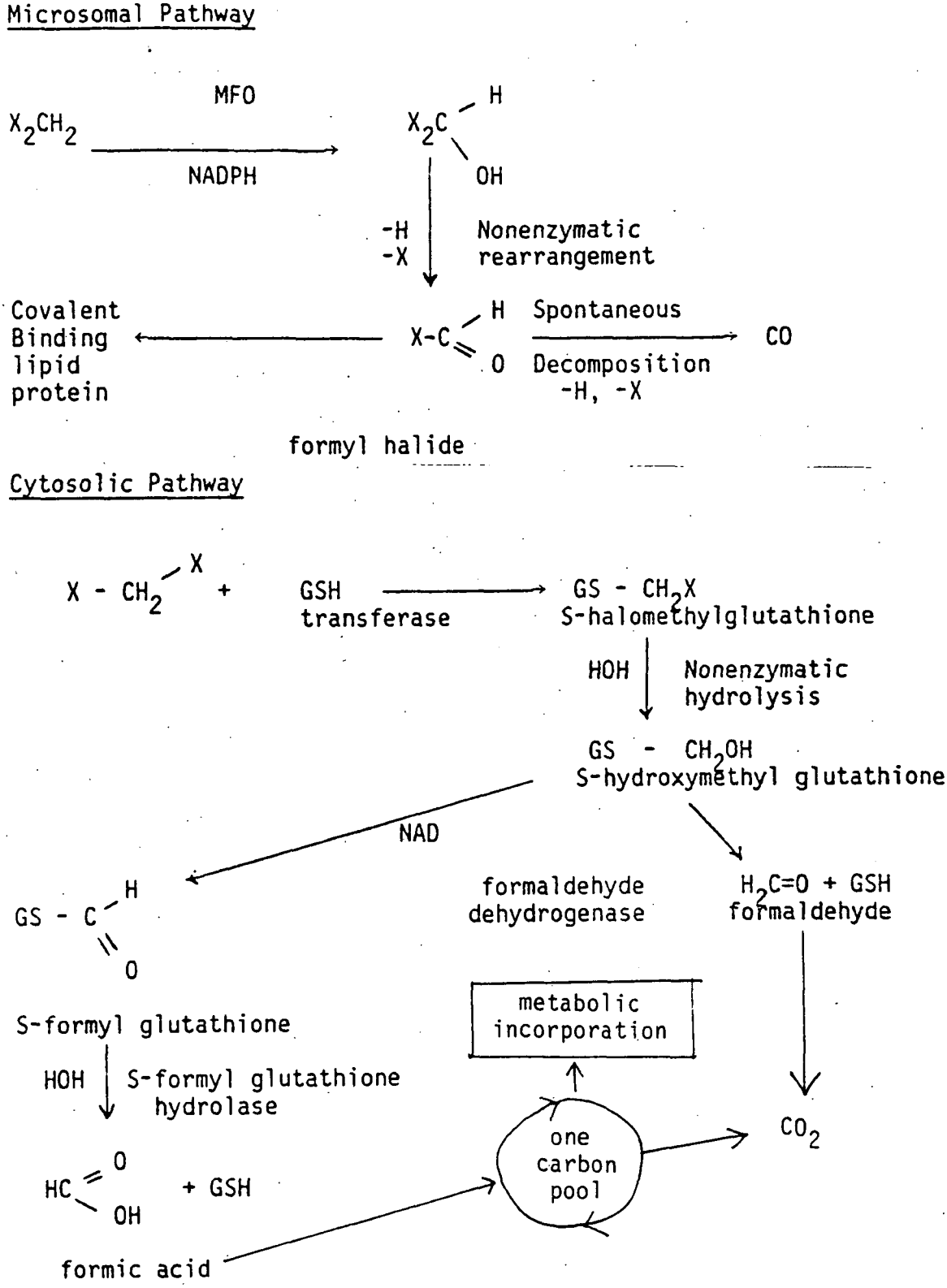

Figure 5. Proposed reaction mechanisms for the metabolism of dihalomethanes to carbon monoxide, carbon dioxide, formaldehyde, formic acid, and inorganic halide.

SOURCE: Ahmed et al., 1980.

of dihalomethanes by liver cytosol is summarized in Figure 5.

3.2.2. Tissue Distribution

There have been a number of studies which have compared DCM metabolism by means of microsomes and/or cytosol prepared from various tissues. Kubic and Anders (1975) compared the biotransformation of DCM to carbon monoxide by liver, kidney, and lung microsomes. They found liver microsomes to be five times more active than lung microsomes, and 30 times more active than kidney microsomes. Ahmed and Anders (1976) compared the biotransformation of DCM to carbon dioxide by cytosolic fractions prepared from various tissues of the rat. The highest activities were found in liver, lung, and kidney cytosol. Liver cytosol was found to be 15 times more active than lung cytosol and 12.5 times more active than kidney cytosol. Ahmed and Anders (1978) measured the metabolism of dibromomethane to bromide, formaldehyde, and formic acid using rat liver cytosol, and observed that free bromide was formed at the rate of 27.7 ± 3.8 nmoles/mg protein/minute, and that the amount of formaldehyde plus formic acid formed was 12.6 ± 0.8 nmoles/mg protein/minute. Kubic and Anders (1975), using rat liver microsomes or cytosol from the same liver preparation, measured dibromomethane metabolism and found that the microsomes yielded 3.9 nmoles carbon monoxide/mg protein/minute and 14.0 nmoles free bromide/mg protein/minute. The cytosol fraction from the same liver preparation converted dibromomethane to bromide at a rate of 9.1 nmoles/mg protein/minute. Based on these data, it appears that similar amounts of DCM/mg protein/unit time are converted to carbon monoxide or carbon dioxide by microsomes and by cytosol. Since microsomes comprise 2% to 5% of liver and cytosol comprises about 10% of liver protein (Estabrook et al., 1971; Hogeboom et al., 1953), it would be anticipated, on a mass basis, that the cytosol would metabolize more DCM than would microsomes. It has been postulated that each pathway

involves the formation of an active intermediate, and each intermediate can be a potential alkylating agent. The available data also indicate that most metabolism of DCM occurs in the liver, although small amounts of activity have been detected in the lung and kidney. Analysis of rat tissues 48 hours post-exposure to ^{14}C-DCM showed that liver, kidney, and lung contain the most radioactivity (McKenna et al., 1982). At the present time, the implications of these observations in assessing the carcinogenic potency of DCM are unclear.

3.2.3. In Vivo Metabolism/Effect of Dose

There have been a number of in vivo studies in which investigators have given animals ^{14}C-DCM and then measured the amount of exhaled ^{14}C-carbon monoxide and ^{14}C-carbon dioxide. Yesair et al. (1977) and Angelo (1985) assessed the metabolism of DCM by mice. Yesair et al. (1977) gave groups of mice 1 mg/kg or 100 mg/kg ^{14}C-DCM in corn oil by intraperitoneal injection, and then collected metabolic by-products for 96 hours. (The authors stated, without giving data, that most of the observed metabolism took place during the first 12 hours post-exposure.) Angelo (1985) gave groups of mice 10 mg/kg or 50 mg/kg ^{14}C-DCM in 25% polyethylene glycol by tail vein injection, and then collected metabolic by-products for 4 hours. The data collected by these investigators are summarized in Table 9. The data show that the mouse is able to metabolize DCM to carbon monoxide and carbon dioxide, and that over a large dose range, equal amounts of DCM are converted to carbon monoxide and carbon dioxide. However, because of different experimental designs used by Yesair et al. (1977) and Angelo (1985), it is not possible to assess whether there is a dose-response relationship. Indeed, the greater conversion of DCM to carbon monoxide and carbon dioxide in mice given 100 mg/kg compared to those given smaller doses is probably an artifact of experimental design, namely the use of corn oil carrier in the intraperitoneal injection of DCM.

TABLE 9. IN VIVO METABOLISM OF DCM BY MICE

Dose	DCM exhaled[a]	% of dose exhaled	CO_2[a]	CO[a]
1 mg/kg[b] (11.76 µmoles/kg)	--		5.9	5.3
10 mg/kg[c] (117.6 µmoles/kg)	56.9	48.4	23.8	17.5
50 mg/kg[c] (588 µmoles/kg)	382.2	65.0	80.6	29.4
100 mg/kg[b] (1,176 µmoles/kg)	470.0	40.0	294.0	235.0

[a]Values are µmoles/kg.
[b]Yesair et al. (1977).
[c]Angelo (1985).

There has been one animal study reported on the metabolism of inhaled ^{14}C-DCM. McKenna et al. (1982) gave groups of rats a single 6-hour inhalation exposure to 50, 500, or 1500 ppm ^{14}C-DCM. At the end of the exposure period, exhaled DCM, carbon dioxide, carbon monoxide, and urine were collected for 48 hours. At the end of the 48-hour collection period, the rats were sacrificed and tissue levels of radioactivity were determined. The data obtained from this study are summarized in Tables 10 and 11 and in Figures 6 and 7. The authors interpreted these data to indicate saturability of metabolism because of the following observations:

- The percent of administered DCM metabolized to carbon dioxide and carbon monoxide declined with increasing dose; and
- There was less than a proportional increase in tissue levels of radioactivity.

The key assumption made by McKenna et al. (1982) was that the disproportionate exhalation of DCM indicated saturated metabolism. However, a review of

TABLE 10. BODY BURDENS AND METABOLIZED ^{14}C-DCM IN RATS AFTER INHALATION EXPOSURE TO ^{14}C-DCM

Exposure concentration	Total body burden,[a] mgEq ^{14}DCM/kg	Metabolized ^{14}DCM[a] mgEq ^{14}DCM/kg	Metabolized ^{14}C-DCM, %
50 ppm	5.53 ± 0.18	5.23 ± 0.32	94.6
500 ppm	48.41 ± 4.33	33.49 ± 0.33	69.2
1,500 ppm	109.14 ± 3.15	49.08 ± 1.37	45.0

[a]Values are mean ± standard deviation; number of animals in each group = 3.

SOURCE: McKenna et al., 1982.

TABLE 11. FATE OF ^{14}C-DCM IN RATS AFTER A SINGLE 6-HOUR INHALATION EXPOSURE

Parameter measured	% body burden ($\bar{x}$ S.D., n = 3)					
	50 ppm		500 ppm		1500 ppm	
Expired CH_2Cl_2	5.42	0.73	30.40	7.10	55.00	1.92
Expired CO_2	26.20	1.21	22.53	4.57	13.61	1.20
Expired CO	26.67	3.00	18.09	0.81	10.23	1.68
Urine	8.90	0.39	8.41	0.90	7.20	0.74
Feces	1.94	0.19	1.85	0.68	2.33	0.05
Carcass	23.26	1.62	11.65	1.87	7.24	0.65
Skin	6.85	1.62	6.72	0.13	3.97	0.15
Cage wash	0.75	0.33	0.24	0.23	0.43	0.15

SOURCE: McKenna et al., 1982.

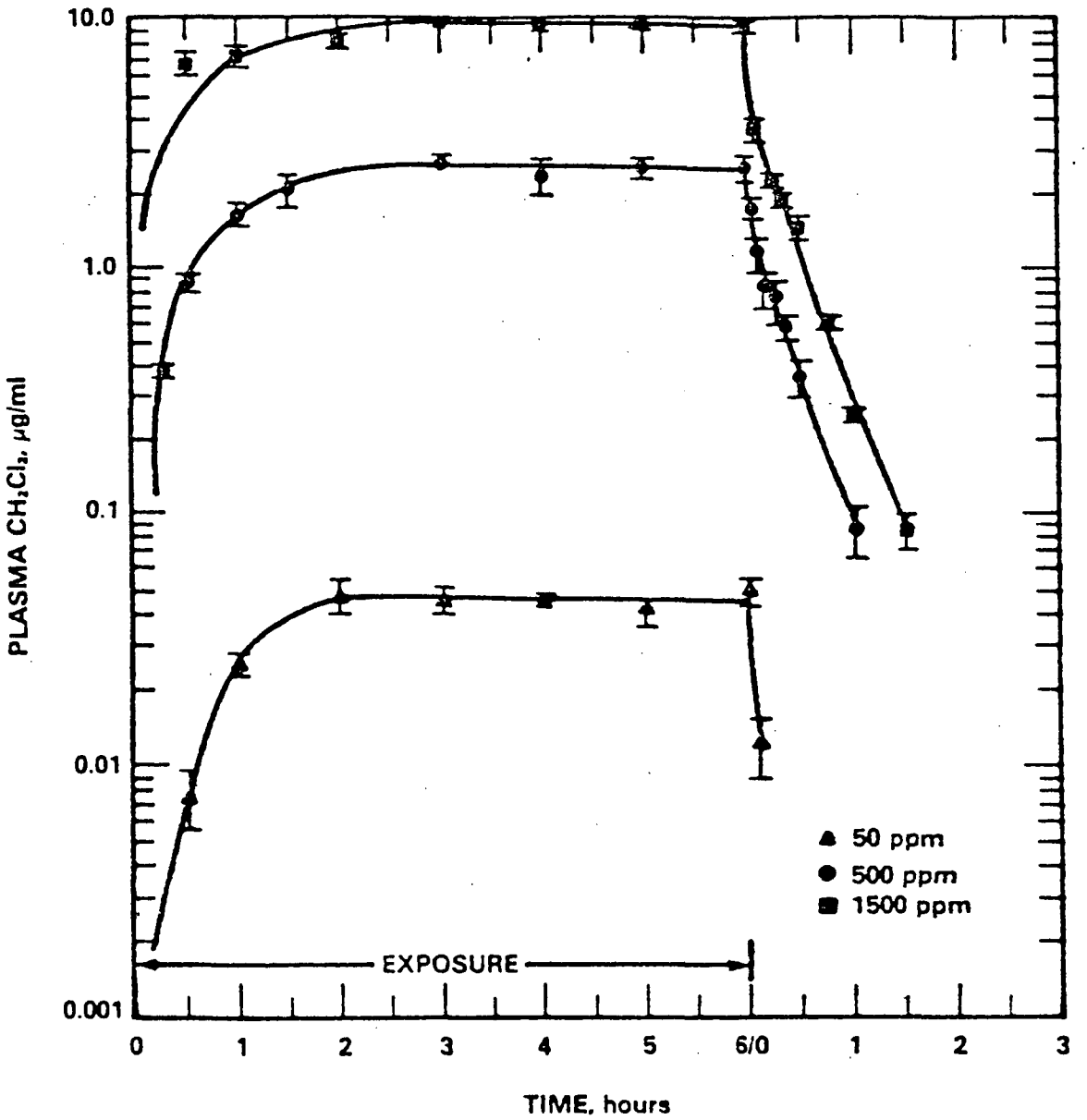

Figure 6. Plasma levels of DCM in rats during and after DCM exposure for 6 hours. Data points represent mean ± standard deviation for two to four rats.

SOURCE: McKenna et al., 1982.

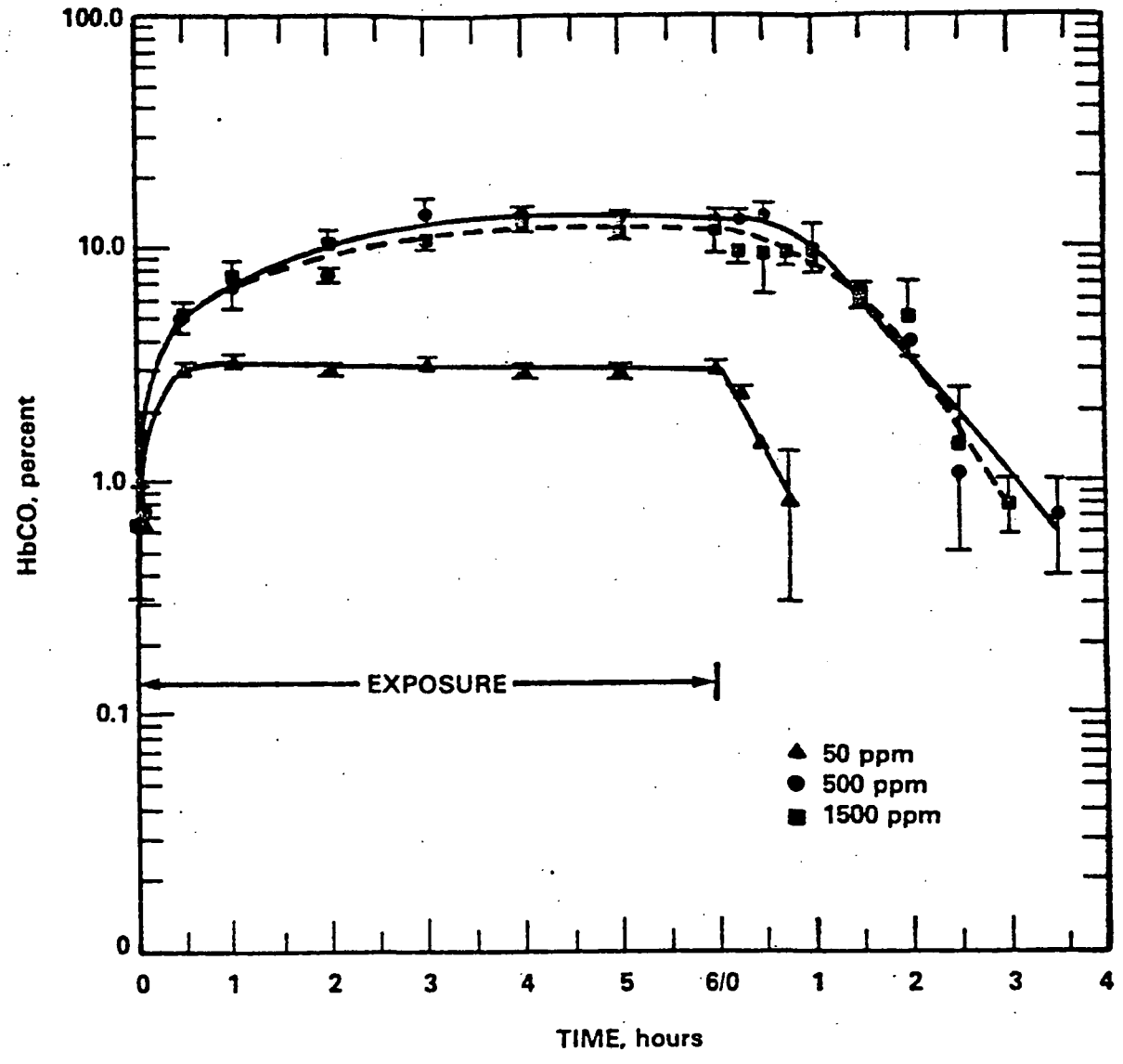

Figure 7. Blood COHb concentrations in rats during and after a 6-hour inhalation exposure to DCM. Each data point is the mean ± standard error for two to four rats.

SOURCE: McKenna et al., 1982.

the study indicates that the data do not support this assumption since the measurements were made when the DCM concentration within the animal was rapidly changing. For the purpose of analysis we selected a three-compartment model (lungs, blood, and tissues). In this model system the DCM concentration within the animal is dependent upon three components: 1) uptake by the lungs via passive diffusion, 2) diffusion into the blood, and 3) uptake by the tissues, metabolism, and exhalation. First, given the volatility of DCM, it is reasonable to expect that exhalation would be the major contributing component in modulating the post-exposure concentration within the animal. Indeed, the very rapid decline in the blood concentration of DCM (10 μg/mL to 0.1 μg/mL in less than 30 minutes) supports this assumption. Thus, the amount of DCM available for metabolism becomes a function of the amount remaining in the tissues post-exposure, and therefore is governed by the air/blood partition coefficient of DCM. While the data reported by McKenna et al. (1982) were not obtained under the steady-state conditions necessary for the comparison of different doses, the study showed that during the exposure period the carboxyhemoglobin (COHb) levels in rats exposed to 500 and 1500 ppm DCM were the same (Figure 7). These data would support the concept that the carbon monoxide pathway was saturated during the period of exposure. However, since similar data on the carbon dioxide pathway were not obtained, it is not possible to conclude that saturated metabolism occurred during the exposure period.

One laboratory has reported results from similar experiments using mice and rats. Angelo (1985) gave 10 mg/kg or 50 mg/kg ^{14}C-DCM intravenously to groups of mice or rats and then measured the amount of exhaled DCM, carbon dioxide, and carbon monoxide for 4 hours post-exposure. The data from these experiments are summarized in Tables 12 and 13. Rats given 10 mg/kg or 50

TABLE 12. METABOLISM OF DCM FOLLOWING INTRAVENOUS ADMINISTRATION OF 10 mg/kg OR 50 mg/kg

Exhaled	Mice		Rats	
	10 mg/kg	50 mg/kg	10 mg/kg	50 mg/kg
DCM	48.4 ± 6.1	65.0 ± 4.2	44.5 ± 3.7	57.6 ± 6.1
Carbon dioxide	20.2 ± 2.0	13.7 ± 1.5	16.0 ± 1.0	9.9 ± 0.7
Carbon monoxide	14.9 ± 4.0	8.4 ± 2.9	12.7 ± 2.2	8.7 ± 2.6

[a]Values are percent of administered dose ± standard deviation.

SOURCE: Angelo, 1985.

TABLE 13. METABOLISM OF DCM FOLLOWING INTRAVENOUS ADMINISTRATION TO CARBON MONOXIDE AND CARBON DIOXIDE

Time post-exposure (min.)	Dose	Mice		Rats	
		Carbon monoxide	Carbon dioxide	Carbon monoxide	Carbon dioxide
0-60	10 mg/kg	0.7[a]	1.7	0.2	0.8
60-240		0.7	0.3	1.1	0.7
Total		1.4	2.0	1.3	1.5
0-60	50 mg/kg	2.1	5.7	0.7	1.9
60-240		2.2	1.2	3.7	3.1
Total		4.3	6.9	4.4	5.0

[a]Values are mg/kg.

SOURCE: Angelo, 1985.

mg/kg immediately (less than 20 minutes) exhaled about 35% of the administered dose, while mice immediately exhaled 45% of the low dose and 60% of the high dose (Table 14). The data, as shown in Table 14, indicate that in both species the amount of DCM metabolized to carbon monoxide and carbon dioxide is not proportional to the increase in administered dose. However, if the administered dose is adjusted by subtracting the amount of DCM immediately exhaled, there is a proportional relationship between dose and metabolites formed in the mouse and the rat.

The adjusted data indicate that on a mg/kg basis the rat and mouse appear to be capable of metabolizing the same amount of DCM over a 4-hour period. This conclusion is supported by the findings of Yesair et al. (1977) and McKenna and Zempel (1981), who assessed the metabolism of small amounts of DCM in mice or rats. Both of the latter studies showed that most of the administered dose was metabolized to carbon monoxide and carbon dioxide.

TABLE 14. METABOLISM OF DCM BY RATS AND MICE
EFFECT OF DOSE CORRECTION FOR EXHALED SUBSTRATE

Species	Dose (mg/kg)	Amount exhaled[a,b]	Adjusted dose[a]	Amount of $CO+CO_2$ formed[a]
Mouse	10	4.6	5.4	3.5
	50	29.8	20.2	11.1
Rat	10	3.2	6.8	2.9
	50	17.6	32.4	9.4

[a]Values are mg/kg.
[b]Amount exhaled in first 20 minutes.

SOURCE: Adapted from Angelo, 1985.

Rodkey and Collison (1977a) exposed a group of 4 rats to 154 µmoles of DCM (1255 ppm) in a chamber having a closed rebreathing system and determined the amount of carbon monoxide exhaled as a function of time. After a short lag period, the rats exhaled carbon monoxide at a rate of 30 µmoles/kg/hour. The authors stated that they obtained similar results after giving animals the same dose of DCM intraperitoneally. In a second experiment, a group of 4 rats exposed to 793 µmoles of DCM (6462 ppm) exhaled 40 µmoles carbon monoxide/kg/hour. These data indicate that in the rat the microsomal pathway is almost saturated at DCM exposures as low as 1255 ppm and that, therefore, the V_{max} equals about 30 to 40 µmoles/kg/hour. Rodkey and Collison (1977b) also measured DCM biotransformation to carbon monoxide and carbon dioxide. Rats were exposed to 200 µmoles of DCM in a chamber with a closed rebreathing system, and the amount of carbon monoxide and carbon dioxide produced were determined at 7 to 9 hours post-exposure. The amount of carbon monoxide formed was about 90 µmoles, whereas the amount of excess carbon dioxide formed was only 56 µmoles. These data appear to differ significantly from those reported by McKenna et al. (1982) and DiVincenzo and Hamilton (1975), who found that more carbon dioxide than carbon monoxide was exhaled. However, since Rodkey and Collison (1977b) accounted for only about 75% of the administered dose, these studies are not directly comparable.

It has been estimated that mice metabolize DCM to carbon monoxide at a rate of about 19 µmoles/kg/hr (EPA, 1985). This value was determined by assuming that the carbon monoxide formation was constant over the 12-hour post-exposure period in the experiments performed by Yesair et al. (1977). Given the volatility of DCM, it is unlikely that the substrate concentration was saturating for 12 hours. Thus the rate constant for carbon monoxide formation in the mouse could well exceed 19 µmoles/kg/hr.

The *in vivo* metabolic studies confirm the data obtained *in vitro* that DCM is biotransformed to carbon monoxide and carbon dioxide. There are some data which indicate that formation of carbon monoxide in the rat reached V_{max} at exposures of less than 1200 ppm. There are no data on the exposure concentration required to saturate the carbon dioxide pathway. Comparative data on species differences and similarities are limited. At low doses both rats and mice metabolize similar amounts of DCM to carbon monoxide and carbon dioxide.

3.2.4. Human Studies

There have been three studies in which volunteers have been exposed to DCM. DiVincenzo and Kaplan (1981a, b) evaluated the conversion of DCM to carbon monoxide in sedentary, non-smoking individuals and in individuals engaged in physical activity. Exposure, in a chamber, was to 50, 100, 150, or 200 ppm DCM for 7.5 hours or for 7.5 hours/day for 5 consecutive days. The metabolism of DCM was also studied in men engaged in physical activity. DiVincenzo and Kaplan (1981a) found that in sedentary individuals the pulmonary uptake of DCM was linear over the range studied (50-200 ppm), and that excretion of carbon monoxide was proportional to the pulmonary uptake of DCM. The authors noted that only 25% to 34% of the DCM taken up was converted to carbon monoxide, and therefore hypothesized, based on data from animal studies, that up to 70% was metabolized to carbon dioxide.

Sedentary volunteers exposed once for 7.5 hours to 50, 100, 150, or 200 ppm of DCM had peak COHb concentrations of 1.9%, 3.4%, 5.3%, and 6.8%, respectively. When sedentary volunteers were exposed to 100, 150, or 200 ppm DCM for 7.5 hours/day for 5 consecutive days, the COHb of those exposed to 150 ppm or 200 ppm increased to levels above that of the single exposure. The volunteer exposed to 200 ppm DCM had a COHb of about 5% on day 1 and a COHb of about 6.5% on day 4. The carbon monoxide concentrations in the breath of

the volunteers also increased each day during exposure to high concentrations (150-200 ppm) of DCM. Conversely, the peak level of expired DCM in the breath of the volunteers remained constant (peak level did not change) throughout the exposure period.

DiVincenzo and Kaplan (1981b) also measured DCM uptake and metabolism during exercise. The data show (see Table 15) that uptake of DCM is directly related to work intensity, and that the amount of DCM metabolized to carbon monoxide is directly proportional to pulmonary uptake. This is illustrated by the finding that a sedentary volunteer exposed to 200 ppm DCM exhaled 6.1 mmoles of carbon monoxide (with a pulmonary uptake 21.1 mmoles DCM), while an exercising volunteer exposed to just 100 ppm DCM exhaled 11.8 mmoles of carbon monoxide (with a pulmonary uptake of 41.9 mmoles DCM). The latter observation suggests that the metabolic capacity of people exposed to 400 ppm DCM would not be exceeded.

McKenna et al. (1980) exposed volunteers to 100 or 350 ppm DCM for 6 hours and measured various parameters, including blood and exhaled air levels of DCM, COHb, and exhaled carbon monoxide. The data showed that the blood level of DCM for both concentrations reached a steady-state in about 2 hours. At the end of the 6-hour exposure, the COHb concentration of the group exposed to 350 ppm DCM was 1.4-fold higher than that of the group exposed to 100 ppm. Likewise, the concentration of exhaled carbon monoxide in the group exposed to 350 ppm DCM was 2.1-fold higher than that of the group exposed to 100 ppm. The authors assumed that once steady-state is achieved, further uptake of DCM would be proportional to the rate of metabolism of DCM. Consistent with this steady-state assumption, McKenna et al. (1980) interpreted these findings to mean that the COHb and carbon monoxide levels between the high and low groups were less than proportional as metabolism became saturated.

TABLE 15. EFFECT OF EXERCISE ON THE PULMONARY UPTAKE AND METABOLISM OF METHYLENE CHLORIDE DURING EXPERIMENTAL EXPOSURES TO METHYLENE CHLORIDE VAPOR

Volunteer	Work intensity (mL O_2 min^{-1} kg^{-1})	Pulmonary uptake of DCM (mmoles)	Total pulmonary excretion of CO (mmoles)
1	4	10.7	2.7
2	14	19.4	5.1
3	15	30.7	10.2
	19	36.4	14.3
4	16	28.8	10.4
	28	41.9	11.8

SOURCE: DiVincenzo and Kaplan, 1981b.

A review of the McKenna et al. (1980) study indicates that the data do not support the assumption that a steady-state blood level of DCM is a reflection of saturated metabolism. The DCM concentration in the blood in the simplest model is a function of three components: uptake by the tissues, metabolism, and exhalation. First, given the volatility of DCM, it is reasonable to expect that exhalation is the major contributing component that modulates the blood level. Indeed, the data from the study support this assumption, since the amount of DCM exhaled increased disproportionately with dose. In addition, the very rapid decline in the blood level of DCM post-exposure argues very strongly that exhalation is the major component governing the observed blood level. Furthermore, the data reported by DiVincenzo and Kaplan (1981a) on volunteers exposed to 50 to 200 ppm DCM clearly show a slight but distinct disproportionate exhalation of DCM when the body mechanism to metabolize DCM

has not been saturated.

The finding by McKenna et al. (1980) that the COHb and carbon monoxide levels in the group exposed to 350 ppm DCM were less than 3.5-fold higher than in the group exposed to 100 ppm, and the subsequent interpretation that this indicates saturated metabolism, ignores the limitations of the design of the experiment. The initial amount of product formed using the same amount of enzyme and two different concentrations of substrate appears to be the same, and if at least one concentration of substrate is less than saturating, the ratio of product formed will continue to change until a steady-state or constant rate of carbon monoxide formation is achieved. The McKenna et al. (1980a) data clearly showed that both the COHb and the concentration of exhaled carbon monoxide concentration were increasing during the exposure portion of the study (Figures 8 and 9), and thus, it is not possible to predict steady-state concentrations of either COHb or exhaled carbon monoxide. Lastly, DCM is metabolized via two pathways. Data from animal studies indicate that both pathways are of equal importance, and that perhaps the carbon dioxide pathway is more important. The authors' conclusion, based on data from only one pathway, that metabolic saturation in humans is achieved at less than 350 ppm DCM is therefore premature.

3.2.5. Summary

The results of both *in vitro* and *in vivo* studies indicate that DCM is metabolized via two pathways. One pathway yields carbon monoxide as an end product, and the other yields carbon dioxide as an end product with formaldehyde and formic acid as metabolic intermediates. Each pathway involves formation of a metabolically active intermediate which is theoretically capable of irreversibly binding to cellular macromolecules. A comparative analysis of the capability of various tissues to metabolize DCM indicates

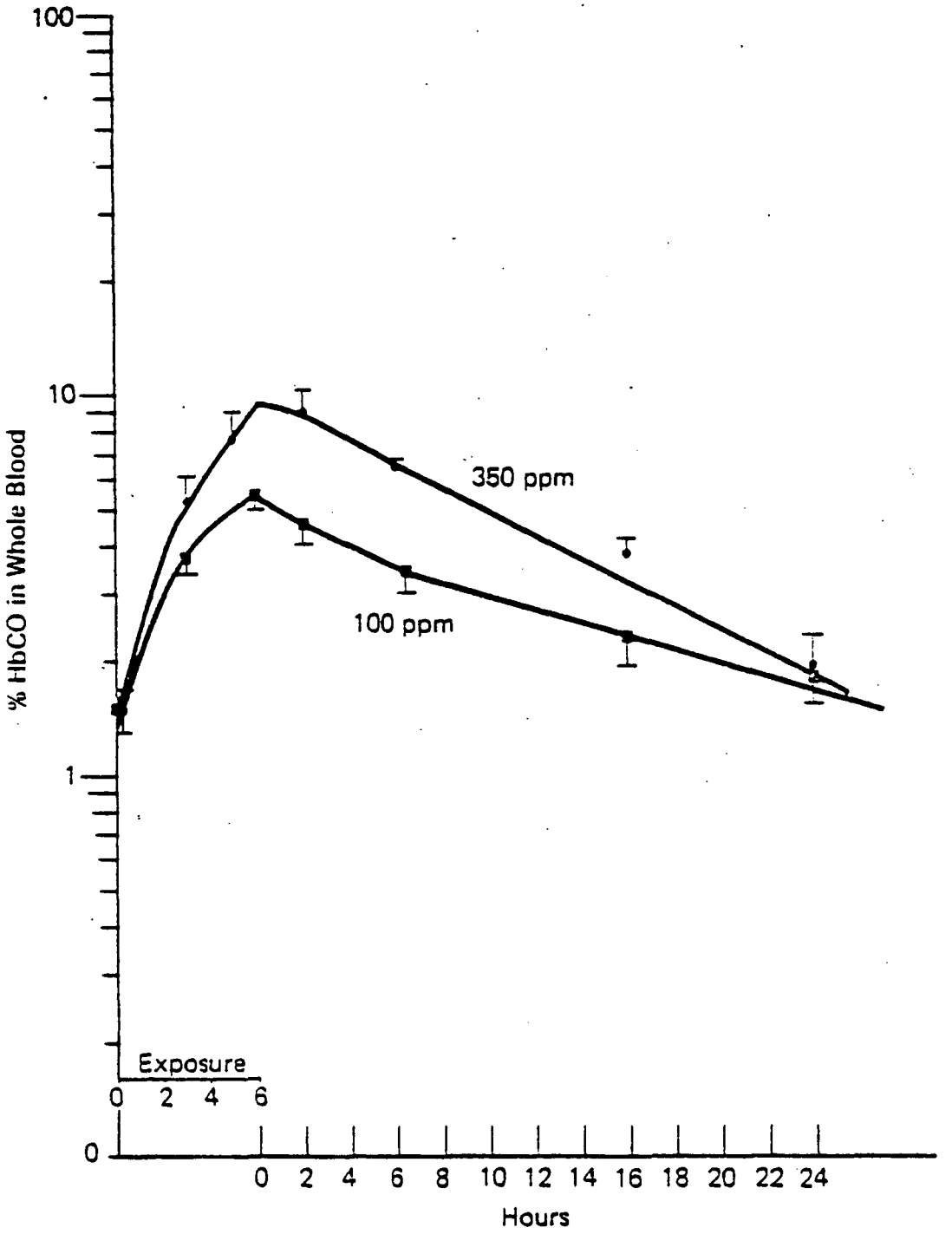

Figure 8. COHb level in volunteers exposed to 100 ppm or 350 ppm DCM.

SOURCE: McKenna et al., 1980.

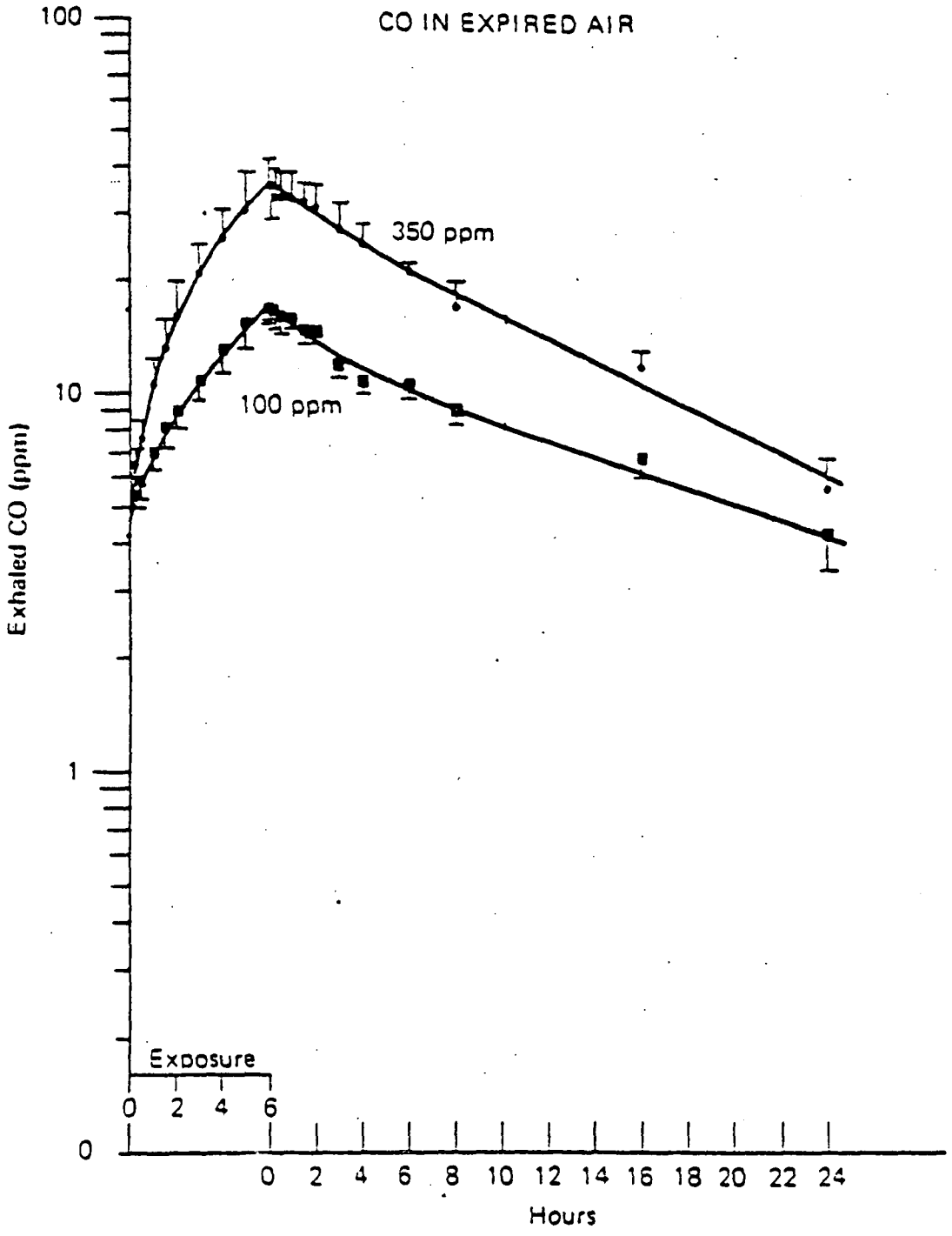

Figure 9. Exhaled carbon monoxide by volunteers exposed to 100 ppm or 350 ppm DCM.

SOURCE: McKenna et al., 1980.

that the liver is the primary site of metabolism, with some metabolism taking place in the lung and kidney. An analysis of the available *in vitro* data suggests that the carbon dioxide pathway may metabolize significantly more DCM than the carbon monoxide pathway. Consistent with this observation is *in vivo* data which suggest that when rats or mice are exposed to high concentrations of DCM they exhale more carbon dioxide and excrete more formic acid than carbon monoxide. At exposure to low concentrations of DCM, both pathways are utilized about equally. The data from rat studies also suggest that the route of exposure, at low doses, results in similar metabolic profiles.

A comparative analysis of the data from *in vivo* studies in mice, rats, and humans indicates that all three species metabolize DCM to carbon monoxide (Table 16). Both mice and rats metabolize DCM to carbon dioxide. There are no human data on the metabolism of DCM to carbon dioxide. However, based on uptake data, some investigators have speculated that this pathway is functional in humans.

Groups of rats exposed to 500 or 1500 ppm DCM have the same COHb, suggesting that the carbon monoxide (microsomal) pathway has been saturated. There are no similar data on the cytosolic pathway. One group of investigators has suggested that the microsomal pathway is saturated in humans at less than 350 ppm DCM. However, an analysis of the study indicates that the data do not support this conclusion.

At present, the available data are insufficient for the purpose of estimating doses of DCM at which metabolism is saturated. The available data indicate that at low doses little unmetabolized DCM is exhaled, and that at high doses there is a significant exhalation of DCM immediately post-exposure. The data suggest that at high doses more DCM is taken up into the body. Currently, there are insufficient data to determine the relationship between

TABLE 16. METABOLISM OF DCM TO CARBON MONOXIDE, CARBON DIOXIDE, AND FORMIC ACID IN MICE, RATS, AND HUMANS

Species	Exposure/uptake	Carbon monoxide (μmoles/kg)	Carbon dioxide (μmoles/kg)
Mice[a]	11.8 μmoles/kg	5.3	5.9
Mice[b]	117.6 μmoles/kg	16.5	22.3
Mice[b]	588.0 μmoles/kg	50.6	81.1
Mice[a]	1176.0 μmoles/kg	235	294
Rats[c]	11.8 μmoles/kg	3.6	4.1 (4.7)[g]
Rats[b]	117.6 μmoles/kg	15.2	17.6
Rats[c]	588.0 μmoles/kg	70	37 (48.8)
Rats[b]	588.0 μmoles/kg	51.7	58.8
Rats[d]	6009.4 μmoles/kg	129.2	182.9 (242.9)
Rats[e]	50 ppm (65.0 μmoles/kg)	17	17.3 (23.1)
Rats[e]	500 ppm (569.3 μmoles/kg)	103	128 (175.8)
Rats[e]	1500 ppm (1,283.5 μmoles/kg)	131	174 (266.4)
Humans[f]	50 ppm (79.1 μmoles/kg)	18.6	55.8
	100 ppm (152.9 μmoles/kg)	28.6	107.1
	150 ppm (219.7 μmoles/kg)	71.4	154.3
	200 ppm (301.0 μmoles/kg)	87.4	210

[a]Yesair et al. (1977). DCM given i.p. in corn oil. According to the authors, the metabolism of methylene was essentially complete 12 hours post-exposure.
[b]Angelo (1985). DCM given i.v. in 25% polyethylene glycol. Metabolism was monitored for 4 hours post-exposure.
[c]McKenna and Zempel (1981). DCM given by gavage. Metabolism was monitored for 48 hours post-exposure.
[d]DiVincenzo and Hamilton (1975). DCM given i.p. in corn oil. Metabolism was monitored for 48 hours post-exposure.
[e]McKenna et al. (1982). DCM given in a single inhalation exposure for 6 hours. Metabolism was monitored for 48 hours post-exposure.
[f]DiVincenzo and Kaplan (1981). DCM given in a single inhalation exposure for 7.5 hours. The amount of post-exposure carbon monoxide exhaled was measured for 24 hours. The amount of carbon dioxide exhaled was based on assumptions made by the authors. For the purpose of comparison, it was assumed that the weight of each volunteer was 70 kg.
[g]Carbon dioxide + radioactivity in urine (assumed to be formic acid).

exposure concentration and uptake. It is of interest to note that the observation has been made that fat people take up more DCM than thin people, and and that high levels of DCM are found in fat tissues post-exposure (U.S. EPA, 1985).

There is a paucity of data on the genotoxicity of DCM. Commercially available DCM gives weak but positive results in Salmonella, yeast, and Drosophila without metabolic activation. It has been shown to induce chromosomal aberrations in some cultured mammalian cell systems but not in others. DCM also causes a weak increase in sister chromatid exchange, but it has not been shown to cause unscheduled DNA synthesis or to inhibit DNA synthesis (U.S. EPA, 1985). At the present time, based on positive mutagenic studies and the likelihood that reactive intermediates are formed during biotransformation to carbon monoxide and carbon dioxide, it seems reasonable to assume that DCM exerts its carcinogenic effect via a genotoxic mechanism.

Based on this analysis, it is concluded that the available pharmacokinetic/metabolism data do not offer useful parameters for making assumptions in the calculation of quantitative carcinogenic risk assessments for DCM.

4. QUANTITATIVE ESTIMATION (USING THE NTP INHALATION BIOASSAY)

The EPA Office of Health and Environmental Assessment has recently published a comprehensive document on the health effects of dichloromethane (methylene chloride, DCM) (U.S. EPA, 1985). After the completion of this report, the NTP released the findings of an inhalation toxicology and carcinogenesis study of DCM in F344/N rats and B6C3F1 mice (NTP, 1985, draft). The qualitative findings of this study are discussed in a preceding section of this document; here, the NTP findings are used to develop estimates of unit incremental cancer risks for humans exposed to DCM. Variations in extrapolated risks using different cancer end points from the NTP study are discussed, as well as the influence of the dose-response model selected. The quantitative findings are also compared with earlier experimental carcinogenesis studies of DCM and with the limited information available from epidemiologic studies.

4.1. SUMMARY OF NTP FINDINGS USED FOR QUANTITATIVE ANALYSIS

In an earlier section of this document, the end points of mammary and subcutaneous tumors in rats and lung and liver tumors in mice were determined to be the sites where the NTP study produced the strongest findings of carcinogenicity for DCM. Tables 17 and 18 present the NTP tumor incidence findings for these sites. The denominator for each data point is the number of animals that were examined at the specific tumor site. The statistical significance of the NTP findings has been discussed earlier in this report, and the findings are only summarized in Tables 17 and 18.

The data in these two tables will serve as the basis for the quantitative risk estimates to be developed. The EPA draft guidelines for carcinogen risk

TABLE 17. SUMMARY OF NTP INHALATION STUDY OF DCM:
FINDINGS FOR MAMMARY AND SUBCUTANEOUS TUMORS IN RATS

Site/tumor	Dose			
	Control	1,000 ppm	2,000 ppm	4,000 ppm
Males				
Mammary gland: adenoma or fibroadenoma[a]	0/50	0/50	2/50	5/50[b]
Subcutaneous tissue: fibroma[c]	1/50	1/50	2/50	4/50
Mammary gland or subcutaneous tissue: adenoma, fibroadenoma or fibroma[a]	1/50	1/50	4/50	9/50[d]
Females				
Mammary gland: fibroadenoma[a]	5/50	11/50[b]	13/50[b]	22/50[d]
Mammary gland: adenoma, fibroadenoma, adenocarcinoma, or mixed tumor, malignant[a]	7/50	13/50[b]	14/50[b]	23/50[d]

[a]All trend tests for tumor incidence positive at $p < 0.01$ nominal level.
[b]One or more positive pairwise comparison(s) with control group $p < 0.05$ nominal level.
[c]One or more positive trend test(s) for tumor incidence $p < 0.05$ nominal level.
[d]All pairwise comparisons with control group positive $p < 0.01$ nominal level.

Note: Tumor trend tests reported by NTP: life table, incidental tumor, and Cochran-Armitage tests; pairwise tests: life table, incidental tumor, and Fisher Exact tests.

SOURCE: NTP, 1985.

TABLE 18. SUMMARY OF NTP INHALATION STUDY OF DCM FINDINGS FOR LUNG AND LIVER TUMORS IN MICE

Tumor	Dose		
	Control	2,000 ppm	4,000 ppm
Males			
Alveolar/bronchiolar adenoma[a]	3/50	19/50[b]	24/50[b]
Alveolar/bronchiolar carcinoma[a]	2/50	10/50[c]	28/50[b]
Alveolar/bronchiolar adenoma or carcinoma[a]	5/50	27/50[b]	40/50[b]
Hepatocellular adenoma[d]	10/50	14/49[c]	14/49[c]
Hepatocellular carcinoma[d]	13/50	15/49	26/49[c]
Hepatocellular adenoma or carcinoma[a]	22/50	24/49	33/49[c]
Alveolar/bronchiolar or hepatocellular carcinoma[e,f]	15/50	21/49	39/49[b]
Alveolar/bronchiolar or hepatocellular adenoma or carcinoma[e,f]	27/50	34/49[d]	45/49[b]
Females			
Alveolar/bronchiolar adenoma[a]	2/50	23/48[b]	28/48[b]
Alveolar/bronchiolar carcinoma[a]	1/50	13/48[b]	29/48[b]
Alveolar/bronchiolar adenoma or carcinoma[a]	3/50	30/48[b]	41/48[b]
Hepatocellular adenoma[a]	2/50	6/48	22/48[b]
Hepatocellular carcinoma[a]	1/50	11/48[b]	32/48[b]
Hepatocellular adenoma or carcinoma[a]	3/50	16/48[b]	40/48[b]
Alveolar/bronchiolar or hepatocellular carcinoma[e,f]	1/50	21/48[b]	43/47[b]
Alveolar/bronchiolar or hepatocellular adenoma or carcinoma[e,f]	5/50	36/48[b]	46/47[b]

[a]All trend tests for tumor incidence positive at $p < 0.01$ nominal level.
[b]All pairwise comparisons with control group positive $p < 0.01$ nominal level.
[c]One or more positive pairwise comparison(s) with control group $p < 0.05$ nominal level.
[d]One or more positive trend test(s) for tumor incidence at $p < 0.05$ nominal level.
[e]Denominators are number of animals examined for tumors at both lung and liver sites.
[f]Tumor grouping not presented in NTP report; significance determined using Fisher Exact Test.

Note: Tumor trend tests reported by NTP: life table, incidental tumor, and Cochran-Armitage tests; pairwise tests: life table, incidental tumor, and Fisher Exact tests.

SOURCE: NTP, 1985.

assessment (U.S. EPA, 1984) call for risk estimation using the combined incidence of statistically elevated tumors. The combined incidence of lung and liver tumors in mice is given for quantitative analysis, and does not imply that the tumors are biologically related.

4.2. DOSE-RESPONSE MODEL SELECTION

The EPA draft guidelines for carcinogen risk assessment (U.S. EPA, 1984) express the fact that there is no rigorously established scientific basis for the selection of a dose-response model to predict carcinogen risks at low doses. In the typical situation where there is limited information on which to base the selection of a model, the draft EPA guidelines express a preference for the multistage dose-response model. The guidelines place emphasis on the upper confidence limit (UCL) risk estimates derived from this model. The bases for the preference include the following:

1) The multistage model incorporates the current scientific opinion that multiple steps are involved in the process of cancer development, and that a chemical carcinogen can contribute to one or more of these steps.

2) The UCL of the multistage model produces a risk estimate that is linear at low dose (LDL). An LDL dose-response is expected when a carcinogen accelerates stages of the carcinogenic process that lead to the background occurrence of cancer in unexposed members of the population.

3) The UCL of the multistage model produces a "plausible upper bound" estimate of risk, i.e., an estimate that is reasonable but is usually as high or higher than estimates derived from other models; models that are not linear at low dose will generally lead to sub-

stantially lower risk estimates.

4) The multistage UCL is stable under small changes in the input values for tumor incidence; in contrast, the maximum likelihood estimate (MLE) can be unstable if the results in one or a few animals are changed.

Because of the role of genetic and mutational factors in the development of many cancers, a supportive biological argument for LDL can be made strongly for chemicals that are known to cause genetic damage. The EPA Health Assessment Document for Dichloromethane (U.S. EPA, 1985) concluded that the weight of evidence shows that DCM is capable of causing gene mutations and has the potential to cause such effects in exposed human cells. Further testing to determine the strength of mammalian evidence was recommended. These findings provide additional support for the application of an LDL dose-reponse model in estimating DCM cancer risks.

Considering both EPA's policy for carcinogen evaluation and the biological information available specifically for DCM, the multistage model was selected as the primary model to be applied in this risk assessment. Versions of the multistage model which incorporate time-to-tumor information have been developed. While time-to-tumor models generally do not produce risk estimates that differ greatly from similar dichotomous models, the results are presented for comparison. Several other models are also presented for comparison with the multistage model.

4.3. APPLICATION OF THE MULTISTAGE MODEL TO NTP BIOASSAY DATA

The multistage dose-response model, as incorporated in the GLOBAL83 computer program developed by Howe (1983), has been applied to the NTP bioassay data given in Section 4.2. The model is applied to the experimentally

rodents exposed to DCM following the same time pattern as the NTP bioassay.

A separate section of this report reviews the available pharmacokinetic and metabolic data on DCM and concludes that these data do not provide an adequate basis for modifications to the experimental doses for use in quantitative risk assessment.

The GLOBAL83 program enables the user to select the highest degree of polynomial that the program will allow in the model. GLOBAL83 runs were made for a polynomial degree equal to the number of doses (counting the control) minus one. Tables 19, 20, 21, and 22 present the results of these computations.

Several conclusions can be drawn from these data.

1) The multistage model provides an adequate fit for all tumor groupings analyzed in both rats and mice.
2) In rats, the highest value for the UCL of the linear multistage term was obtained for mammary tumors in female rats. The highest value in males was lower by a factor of three.
3) In mice, the highest value for the UCL of the linear term was obtained in females having either adenomas or carcinomas of the lung and/or liver. The corresponding value for males was lower by a factor of two.
4) In mice, the male and female high-dose groups had a high percentage of tumors in both the lung and liver. As discussed in Section 4.4., the high-dose mice also showed elevated mortality in comparison to controls. In these circumstances, competing risks can lead to underestimates of risk attributable to individual tumor types.
5) The NTP (1985) noted that all male rat groups (including controls)

TABLE 19. GLOBAL83 MODEL PARAMETERS FOR NTP (1985) RAT DCM DATA

Site	3-stage model				
	c_0	$q_1 \times 10^{-3}$ (ppm^{-1})	$q_2 \times 10^{-6}$ (ppm^{-2})	$q_3 \times 10^{-10}$ (ppm^{-3})	$q_1^* \times 10^{-3}$ (ppm^{-1})
Males					
Mammary gland[a]	0.0	0.0	0.00696	0.0	0.0311
Subcutaneous[b]	0.0191	0.00093	0.00389	0.0	0.0306
Mammary gland or subcutaneous[c]	0.0197	0.00075	0.0117	0.0	0.0540
Females					
Mammary gland[d]	0.111	0.107	0.0	0.00544	0.164
Mammary gland[e]	0.161	0.0985	0.0	0.00846	0.164

[a]Adenoma or fibroadenoma.
[b]Fibroma.
[c]Adenoma, fibroma, or fibroadenoma.
[d]Fibroadenoma.
[e]Adenoma, fibroadenoma, adenocarcinoma, or mixed tumor, malignant.
q_1 = The i^{th} power coefficient in the multistage model.

q_1^* = The 95% upper confidence limit estimate of the linear coefficient.

Values for the q_i apply for rats exposed under the NTP protocol dose time schedule.

Calculations are based on an extra risk analysis.

TABLE 20. COMPARISON OF THREE-STAGE GLOBAL83 ESTIMATES WITH OBSERVED TUMOR RESPONSE - RATS

Tumor	Observed response (%)				Predicted response (%)				Chi squared
	Control	1,000 ppm	2,000 ppm	4,000 ppm	Control	2,000 ppm	4,000 ppm	4,000 ppm	
Males									
Mammary gland adenoma or fibroadenoma	0.0	0.0	4.0	10.0	0.0	0.7	2.7	10.5	0.66
Subcutaneous	2.0	2.0	4.0	8.0	1.9	2.4	3.6	8.2	0.06
Mammary gland or subcutaneous	2.0	2.0	8.0	18.0	1.8	3.0	6.4	18.8	0.42
Females									
Mammary gland fibroadenoma	10.0	22.0	26.0	44.0	10.5	19.7	28.1	43.7	0.30
Mammary gland, all tumors	14.0	26.0	28.0	46.0	14.9	22.9	30.6	45.6	0.46

TABLE 21. GLOBAL83 MODEL PARAMETERS FOR NTP (1985) MOUSE DCM DATA

Tumor	Two-stage model			
	q_0	$q_1 \times 10^{-3}$ (ppm^{-1})	$q_2 \times 10^{-6}$ (ppm^{-2})	$q_1^* \times 10^{-3}$ (ppm^{-1})
Males				
Lung[a] adenoma	0.0672	0.166	0.0	0.223
Lung carcinoma	0.0398	0.0	0.0481	0.141
Lung adenoma or carcinoma	0.105	0.295	0.0202	0.452
Liver[b] adenoma	0.237	0.0306	0.0	0.0810
Liver carcinoma	0.285	0.0	0.0283	0.142
Liver adenoma or carcinoma	0.565	0.0	0.0338	0.195
Lung or liver carcinoma	0.333	0.0	0.0739	0.190
Lung or liver adenoma or carcinoma	0.771	0.0	0.107	0.429
Females				
Lung adenoma	0.0445	0.242	0.0	0.310
Lung carcinoma	0.0202	0.0690	0.0394	0.233
Lung adenoma or carcinoma	0.0619	0.453	0.0032	0.579
Liver adenoma	0.0356	0.0	0.0336	0.0872
Liver carcinoma	0.0193	0.0	0.0655	0.145
Liver adenoma or carcinoma	0.0576	0.0	0.101	0.160
Lung or liver carcinoma	0.0197	0.0	0.147	0.244
Lung or liver adenoma or carcinoma	0.105	0.345	0.148	0.870

[a]Lung = alveolar/bronchiolar.
[b]Liver = hepatocellular.
[c]Only the linear parameters are given for the 4-stage model.
q_i = The i^{th} power coefficient in the multistage model.

q_1^* = The 95% upper confidence limit estimate of the linear coefficient.

Values for the q_i apply for mice exposed under the NTP protocol dose time schedule.

Calculations are based on an extra risk analysis.

TABLE 22. COMPARISON OF TWO-STAGE GLOBAL83 ESTIMATES WITH OBSERVED TUMOR RESPONSE - MICE

Tumor	Observed response (%)			Two-stage prediction (%)			Chi square
	Control	2,000 ppm	4,000 ppm	Control	2,000 ppm	4,000 ppm	
Females							
Lung adenoma	4.0	47.9	58.3	4.3	41.0	63.7	1.54
Lung carcinoma	2.0	27.1	60.4	2.0	27.1	60.4	<.01
Lung carcinoma or adenoma	6.0	62.5	85.4	6.0	62.5	85.4	<.01
Liver adenoma	4.0	12.5	45.8	3.5	15.6	43.6	0.49
Liver carcinoma	2.0	22.9	66.7	1.9	24.5	65.6	0.93
Liver carcinoma or adenoma	6.0	33.3	83.3	5.6	37.0	81.3	0.43
Liver/lung carcinoma	2.0	43.8	91.5	2.0	45.5	90.6	0.10
Liver/lung carcinoma or adenoma	10.0	75.0	97.9	10.0	75.0	97.9	<.01
Males							
Lung adenoma	6.0	38.0	48.0	6.5	33.0	52.0	0.90
Lung carcinoma	4.0	20.0	56.0	3.9	20.7	55.5	0.02
Lung carcinoma or adenoma	10.0	54.0	80.0	10.0	54.0	80.0	<.01
Liver adenoma	20.0	28.6	28.6	21.1	25.8	30.2	0.30
Liver carcinoma	26.0	30.6	53.1	24.8	32.8	52.2	0.16
Liver carcinoma or adenoma	44.0	49.0	67.3	43.1	50.3	66.9	0.06
Lung/liver carcinoma	30.0	42.9	79.6	28.3	46.6	78.0	0.42
Lung/liver carcinoma or adenoma	54.0	69.4	91.8	53.7	69.9	91.7	0.08

experienced higher than usual mortality before final sacrifice. In female rats, there was increased mortality in the high-dose group relative to controls. Under these conditions, competing risks may lead to underestimation of the risk attributable to the tumors observed in the rats. The NTP indicated that the decreased rat survival is likely to be due to the frequent occurrence of leukemia in all groups.

The draft EPA carcinogen evaluation guidelines (U.S. EPA, 1984) indicate that weight should be placed on the analysis of risks using the animals that have tumors in any one of the sites found to be statistically elevated. The draft guidelines also indicate that weight should be placed on the experimental species and sex group showing the highest risks. On these grounds, combined carcinomas and adenomas of the lung and/or liver in female mice is the end point of most weight. Thus, additional analyses were conducted, with emphasis on the data for mice having lung and/or liver tumors. The following sections describe these analyses.

4.4. RISK ANALYSIS CONSIDERING TIME-TO-TUMOR INFORMATION

In the NTP study, there was a small number of deaths in mice before the first lung or liver tumors were found. The first lung or liver tumor was a liver tumor in a high-dose male mouse at week 61. Table 23 shows the numbers of male and female mice alive at 61 weeks that were subsequently examined at the lung and liver sites.

While the number of animals lost to early mortality was small, because of the very high incidence of tumor responses seen in the high-dose groups, analyses were conducted to determine if the early deaths might have an effect on estimated risks. Table 24 shows the results of applying the two-stage multistage model to the mouse data using the denominators from Table 23.

TABLE 23. MICE SURVIVING TO 61 WEEKS AND RECEIVING EXAMINATION OF THE LUNG AND LIVER

Response	Dose		
	Control	2,000 ppm	4,000 ppm
Males	50	45	47
Females	46	46	46

Note: In all, 13 mice were excluded from counting due to early death. The times of these deaths were: 0-3 months, 5 deaths; 3-6 months, 1 death; 6-9 months, 4 deaths; 9-12 months, 1 death; 12 months-61 weeks, 2 deaths.

SOURCE: NTP, 1985.

TABLE 24. GLOBAL83 MODEL PARAMETERS FOR MOUSE LUNG AND LIVER TUMORS COMBINED--NTP (1985) DCM DATA ON ANIMALS SURVIVING TO 61 WEEKS (WHEN FIRST TUMOR OCCURRED)

Tumor	Two-stage model parameters			
	q_0	$q_1 \times 10^{-3}$ (ppm^{-1})	$q_2 \times 10^{-6}$ (ppm^{-2})	$q_1^* \times 10^{-3}$ (ppm^{-1})
Males				
Lung or liver carcinoma	0.341	0.0	0.0854	0.235
Lung or liver carcinoma or adenoma	0.777	0.0371	0.139	0.582
Females				
Lung or liver carcinoma	0.0211	0.0	0.159	0.245
Lung or liver carcinoma or adenoma	0.114	0.0	0.363	0.785

Values of the q_i apply for the NTP protocol dose schedule.

It should be noted that this simplified analysis has the weakness that animals dying before the first tumor was observed may in reality (as would be seen in a large population) have experienced some risk of cancer, and secondly, that animals dying after the first tumor was observed are treated as having been at full lifetime risk of cancer independent of their time of death.

The data in Table 24, in comparison with the data in Table 21, show that the GLOBAL q_1^* parameter estimates are consistent with the earlier analysis and are not strongly influenced by the adjustment of the denominators. In contrast, for some of the analyses, the MLE q_1 estimates are unstable: for male mice the q_1 estimate for combined adenomas or carcinomas of the lung or liver in Table 21 is zero, whereas in Table 24 it is positive. For female mice, for the same combination of tumors and sites, this situation is reversed. The variability of the MLE linear term is consistent with the CAG's experience that this parameter estimate is often unstable in response to small changes in the input data.

To provide a comparison with the dichotomous multistage model risk estimates developed in the preceding section, a time-to-tumor formulation of the multistage model, the WEIBULL82 time-to-tumor program, also developed by Crump (19__), was applied to NTP cancer results in mice. The WEIBULL82 program is based on the following equation:

$$\text{Prob [effect]} = 1 - \exp\left[-Q\,(\text{dose}) \times (\text{time} - T_0)^K\right]$$

where Q is a fitted polynomial of the same form utilized in GLOBAL83, and T_0 and K are fitted parameters for the time-dependence of the tumor response. Risk calculations using this model can be made for two end points: either tumors are assumed to be "incidental" and unrelated to the cause of an animal's

death, or the tumors are "fatal" and are assumed to produce death directly. NTP studies do not attempt to identify the cause of death for animals in cancer bioassays; therefore, a time-to-tumor analysis must hypothesize as to the causes of deaths in study animals dying before final sacrifice. The NTP (1985) DCM bioassay report provides statistical analyses showing that in mice both the male and female high-dose groups experienced elevated mortality in the latter part of the 2-year study. A simple comparison demonstrates that the observed tumors may reasonably have produced this mortality. Table 25 shows the study mortality divided into three time periods, deaths before 61 weeks (when the first lung or liver tumor was observed), deaths between 61 and 103 weeks, and deaths at the final sacrifice at 104 weeks. The table also indicates the numbers of animals, in each death category, which were observed to have carcinomas of either the lung or liver; these are the tumor types that can be most strongly expected to contribute to mortality. Table 26 shows that mortality before the occurrence of the first lung or liver tumor was small and comparable in all groups. In the 61-103 week period, where elevated treatment-associated mortality is seen, the number of animals dying without carcinomas was relatively stable among the male dose groups. In high-dose females, few animals died without carcinomas. These data are consistent with DCM-induced tumors leading to the increased mortality observed in the NTP mice.

In the following analysis, using the WEIBULL82 program, the effect of the alternate assumptions of incidental or fatal tumors will be compared. For completeness, both "fatal" and "incidental" analyses are presented for adenoma response as well as for the other tumor groupings. However, it is noted that a "fatal" tumor analysis may be less appropriate for adenomas. The data from these analyses are given in Table 26.

TABLE 25. MORTALITY IN NTP MOUSE DCM BIOASSAY

Dose	Deaths before 61 weeks	Deaths 61-103 weeks	Final sacrifice deaths
Males			
Control	0(0)[a]	11(5)	39(10)
2,000 ppm	4(0)	22(10)	24(11)
4,000 ppm	3(0)	36(29)	11(10)
Females			
Control	4(0)	21(0)	25(1)
2,000 ppm	4(0)	21(8)	25(13)
4,000 ppm	4(0)	38(35)	8(8)

[a]Numbers in parentheses indicate the number of animals in each group found to have lung or liver carcinomas.

SOURCE: NTP, 1985.

TABLE 26. COMPARISON OF WEIBULL82 AND GLOBAL83 PREDICTIONS FOR RISK AT 104 WEEKS--MICE LUNG AND LIVER TUMORS[a]

Tumor	"Fatal" tumor WEIBULL82 analysis $q_1 \times 10^{-3}$ (ppm^{-1})	"Fatal" tumor WEIBULL82 analysis $q_1^* \times 10^{-3}$ (ppm^{-1})	"Fatal" tumor WEIBULL82 analysis n[b]	"Incidental" tumor WEIBULL82 analysis $q_1 \times 10^{-3}$ (ppm^{-1})	"Incidental" tumor WEIBULL82 analysis $q_1^* \times 10^{-3}$ (ppm^{-1})	"Incidental" tumor WEIBULL82 analysis n	GLOBAL83 Two-stage $q_1 \times 10^{-3}$ (ppm^{-1})	GLOBAL83 Two-stage $q_1^* \times 10^{-3}$ (ppm^{-1})
Males								
Lung adenoma	0.125	0.173	5	0.232	0.299	5	0.166	0.223
Lung carcinoma	--	--	-	0.0561	0.199	5	0.0	0.141
Liver adenoma	0.0344	0.0787	5	0.0687	0.131	5	0.0306	0.0810
Liver carcinoma	0.0417	0.1502	5	0.0369	0.159	5	0.0	0.142
Lung or liver carcinoma	0.0573	0.199	4	0.0526	0.304	4	0.0	0.190
Lung or liver carcinoma or adenoma	0.106	0.252	4	0.178	0.644	4	0.0	0.429
Females								
Lung adenoma	0.0	0.148	2	0.352	0.447	4	0.242	0.310
Lung carcinoma	<.001	0.0651	4	0.109	0.248	4	0.690	0.233
Liver adenoma	0.0	0.0440	4	0.0084	0.105	4	0.0	0.0872
Liver carcinoma	0.002	0.0975	4	0.0526	0.198	4	0.0918	0.145
Lung or liver carcinoma	0.0153	0.137	4	0.119	0.392	4	0.0	0.244
Lung or liver carcinoma or adenoma	0.0769	0.210	4	0.0	0.822	4	0.345	0.870

[a]For the WEIBULL82 model, q_1 is defined as the first-degree polynomial coefficient multiplied by the time function evaluated at week 104. q_1^* is derived from model risk predictions at low dose.
[b]n indicates the degree of the dose polynomial used in the WEIBULL82 analysis. Different degrees were used in this exploratory analysis.

Values of the q_i apply for the NTP protocol dose schedule.

The following observations can be drawn from these data:

1) The q_1 and q_1^* values for the "incidental" tumor analysis from the WEIBULL83 program were generally higher than the values from the "fatal" tumor analysis. However, the size of this difference was moderate, constituting approximately a factor of two, with a maximum difference of a factor of four for q_1^*. Such differences are expected because the "fatal" tumor analysis excludes tumors found at final sacrifice, and these tumors provide important quantitative contributions to the NTP findings (see Table 25 for examples). While the q_1 estimates generally followed this same pattern, the deviations were greater in some cases.

2) q_1^* and q_1 estimates from the two-stage GLOBAL83 analysis agreed overall with the range of "incidental" and "fatal" tumor WEIBULL82 analyses. In all cases, the GLOBAL83 q_1^* values fell either within or very close to the range of the two WEIBULL q_1^* values.

From this exploratory analysis, it can be seen that use of the WEIBULL82 time-to-tumor analysis does not lead to risk estimates strongly different from those derived from GLOBAL83. The WEIBULL82 program has been less widely utilized than the GLOBAL83 program, and requires assumptions as to the cause of animal death. For these reasons, WEIBULL82 is less well suited than GLOBAL83 for formal use in the present risk assessment.

4.5. COMPARISON OF RISKS ESTIMATED WITH OTHER DOSE-RESPONSE MODELS

In order to provide comparison with the two-stage (restricted) multistage estimates presented in Table 5, calculations for combined lung and liver tumors were also made with the one-stage (one-hit) and four-stage (dichotomous) formulations of the multistage model. The one-stage or linear model has been one of the most widely used models in carcinogen risk assess-

ment, and provides a simple formulation of the hypothesis that many fundamental carcinogenic processes are linear in nature. The four-stage model shares the multistage rationale of the two-stage model, but allows a sharper upward curvature in risk estimates. Table 27 shows parameter estimates for one-stage and four-stage versions of the GLOBAL83 program for tumor response data from Table 18. The one-stage model fits the experimental data acceptably for the combined carcinomas and adenomas in both the male and female groups. For the females, the one-stage model response estimates are within 4% of the observed response for the three experimental doses; for males, the estimates are within 6% of the observed response. For carcinomas alone, the one-hit model does not provide a good fit to the data in either sex ($p < 0.05$ males, $p < 0.01$ females, by the chi square test). As can be seen from Tables 21 and 22, the two-stage model provides an acceptable fit to the data for all four tumor end points. The four-stage model provides an exact fit for all four data sets.

These analyses demonstrate that for combined adenomas and carcinomas of the lung and/or liver in both male and female mice, the data are compatible with a linear dose-response, and that the 95% UCL linear term estimates from the three models are consistent within 20% in males and within 10% in females. For the carcinoma response, where the one-stage model did not fit well, the four-stage model yielded positive MLE linear term estimates for both sexes, while the two-stage model did not. The UCL four-stage risk estimates were approximately 50% and 20% higher than the UCL two-stage estimates for combined lung and liver carcinomas in the males and females, respectively.

To provide comparisons with multistage risk model estimates, two models with different theoretical formulations, the Weibull (dichotomous) and probit models, were used to analyze the combined lung and liver tumor data in both the male and female mice. These models were fitted to the data using the RISK81

TABLE 27. ONE-STAGE AND MULTISTAGE MODEL PARAMETERS FOR TUMORS IN MICE[a]

Tumor	One-stage			Two-stage			Four-stage		
	q_1	q_1^*	Chi square	q_1	q_1^*	Chi square	q_1	q_1^*	Chi square
Male mice									
Lung or liver carcinoma	0.238	0.329	4.44	0.0	0.190	0.42	0.058	0.223	<.01
Lung or liver carcinoma or adenoma	0.348	0.505	1.94	0.0	0.424	<.01	0.125	0.429	<.01
Female mice									
Lung or liver carcinoma	0.418	0.522	6.77	0.0	0.244	<.01	0.120	0.368	<.01
Lung or liver carcinoma or adenoma	0.736	0.936	1.25	0.345	0.870	<.01	0.472	0.870	<.01

[a]Units: $ppm^{-1} \times 10^{-3}$.

computer program developed by Kovar and Krewski (1981). The RISK81 program provides two formulations of both models, one which is based on the assumption that the observed tumor incidence is independent of the background tumor rates observed in the controls, and a second formulation which assumes that the carcinogen contributes a dose that is additive to the background effects seen in the controls. In the additive case, the probit and Weibull models produce risk estimates that are linear at low dose.

Tables 28 through 31 provide the results from these models in comparison to the two-stage GLOBAL83 results. The tables show the DCM doses that are estimated to produce four given levels of risk under the different models, as well as lower confidence limits (LCLs) on the dose that produces the specified effect. LCL dose estimates correspond to UCL risk estimates.

In all cases, the doses estimated to produce a given risk by the background-independent probit model are markedly higher than those predicted by the multistage model (four orders of magnitude difference in the female mice combined tumor group). While the independent Weibull MLE estimates of dose are substantially below those obtained with the independent probit model, they are substantially higher than those obtained with the two-stage multistage model in all four analyses. The independent Weibull MLE estimates are broadly comparable to the multistage MLE estimates for cases where the MLE multistage linear term is zero. The LCL dose estimates of the independent Weibull model are notably lower than the MLE estimates.

The additive background formulation of both the Weibull and probit models converged to provide acceptable parameter estimates only for the female mouse data sets. In these two cases, both models produced estimates of dose for a fixed risk that were linear at low dose and were markedly higher than the corresponding independent background models. The MLE and LCL dose estimates

TABLE 28. MALE MICE CARCINOMAS OF LUNG OR LIVER:
DCM DOSES ESTIMATED TO PRODUCE GIVEN RISK LEVELS
(DOSES IN PPM UNDER NTP PROTOCOL SCHEDULE)

	GLOBAL83 two-stage		Independent probit		Independent Weibull	
Risk level	MLE	LCL	MLE	LCL	MLE	LCL
10^{-2}	369	52.4	1,080	531	723	217
10^{-4}	36.8	0.524	543	185	123	11.0
10^{-6}	3.68	5.24×10^{-3}	329	84.7	20.9	0.556
10^{-8}	0.368	5.24×10^{-5}	217	44.4	3.57	0.0281

Calculations are based on excess risk over background.
Probit and Weibull model estimates calculated using RISK81 computer program.
LCL estimates are the 95% confidence lower bound on dose (variance based on log dose for RISK81).

Note: The additive probit and additive Weibull models failed to converge for this data set.

TABLE 29. MALE MICE CARCINOMA OR ADENOMA OF LUNG OR LIVER:
DCM DOSES ESTIMATED TO PRODUCE GIVEN RISK LEVELS
(DOSES IN PPM UNDER NTP PROTOCOL SCHEDULE)

	GLOBAL83 two-stage		Independent probit		Independent Weibull	
Risk level	MLE	LCL	MLE	LCL	MLE	LCL
10^{-2}	306	23.4	885	353	493	100
10^{-4}	30.6	0.233	410	101	54.0	2.09
10^{-6}	3.06	2.33×10^{-3}	236	41.0	5.93	0.0439
10^{-8}	0.306	2.33×10^{-5}	150	19.5	0.652	9.16×10^{-4}

Calculations are based on excess risk over background.
Probit and Weibull model estimates calculated using RISK81 computer program.
LCL estimates are the 95% lower confidence limits on dose (variance based on log dose for RISK81).

Note: The additive probit and additive Weibull models failed to converge for this data set.

TABLE 30. FEMALE MICE CARCINOMAS OF LUNG OR LIVER:
DCM DOSES ESTIMATED TO PRODUCE GIVEN RISK LEVELS
(DOSES IN PPM UNDER NTP PROTOCOL SCHEDULE)

Risk level	GLOBAL83 two-stage		Independent probit		Additive probit		Independent Weibull		Additive Weibull	
	MLE	LCL	MLE	LCL	MLE	LCL	MLE	LCL	MLE	LCL
10^{-2}	262	40.7	769	503	163	81.4	309	142	134	68.8
10^{-4}	26.1	0.410	412	218	1.92	0.78	35.8	8.03	1.52	0.686
10^{-6}	2.61	4.10×10^{-3}	260	117	0.0192	0.0078	4.15	0.452	0.0152	0.00686
10^{-8}	0.261	4.10×10^{-5}	176	70.1	1.92×10^{-4}	7.8×10^{-5}	0.482	0.0255	1.52×10^{-4}	6.86×10^{-5}

Calculations are based on excess risk over background.
Probit and Weibull model estimates calculated using RISK81 computer program.
LCL estimates are the 95% lower bound on the dose (variance based on log dose for RISK81).

TABLE 31. FEMALE MICE CARCINOMA OR ADENOMA OF LUNG OR LIVER:
DCM DOSES ESTIMATED TO PRODUCE GIVEN RISK LEVELS
(DOSES IN PPM UNDER NTP PROTOCOL SCHEDULE)

Risk level	GLOBAL83 two-stage		Independent probit		Additive probit		Independent Weibull		Additive Weibull	
	MLE	LCL	MLE	LCL	MLE	LCL	MLE	LCL	MLE	LCL
10^{-2}	28.8	11.6	478	198	45.1	24.6	93.4	17.2	36.3	15.7
10^{-4}	0.290	0.115	238	67.4	0.464	0.249	4.75	0.196	0.369	0.156
10^{-6}	2.90×10^{-3}	1.15×10^{-3}	141	30.2	4.64×10^{-3}	2.49×10^{-3}	0.242	2.23×10^{-3}	3.69×10^{-3}	1.56×10^{-3}
10^{-8}	2.90×10^{-5}	1.15×10^{-5}	92.5	15.6	4.64×10^{-5}	2.49×10^{-5}	0.0124	2.53×10^{-5}	3.69×10^{-5}	1.56×10^{-5}

Calculations are based on excess risk over background.
Probit and Weibull model estimates calculated using RISK81 computer program.
LCL estimates are the 95% lower bound on dose (variance based on log dose for RISK81).

were quite comparable between the two models (maximum differences under 50% at low dose). For female mouse carcinomas, the MLE estimates from these models lead to dose estimates that are markedly lower than the (non-linear) MLE multistage estimates. For the combined carcinomas and adenomas, the MLE estimates of additive Weibull, additive probit, and multistage models agreed within approximately 50%. For both groupings of tumors in female mice, the LCL dose estimates of the additive probit, additive Weibull, and multistage models were within a factor of two of each other, with the two-stage multistage model leading to the lower estimates of dose for a fixed risk.

4.6. COMPARISON OF NTP (1985) RESULTS WITH OTHER BIOASSAYS

There are several recent long-term animal studies of DCM in addition to the NTP (1985) bioassay. These studies are reviewed in detail in the EPA Health Assessment Document for DCM (U.S. EPA, 1985). While there were significant limitations in these studies, which often included maximum doses well below what the animals could tolerate, some positive and suggestively positive carcinogenic responses were obtained. This section develops GLOBAL83 parameter estimates (for a multistage model with the maximum polynomial degree equal to the number of dose groups minus one) for the responses found in rats and mice, using data taken from EPA (1985).

1) The Dow Chemical Company (1980) 2-year inhalation study in Sprague-Dawley rats found some statistically positive elevations in mammary tumors in this strain, which has a normally high spontaneous mammary tumor rate, making positive results more difficult to obtain statistically. The strongest finding was the elevation of the total number of mammary tumors seen in female rats (165/99 in controls vs. 287/97 in the high-dose groups). However, the GLOBAL83 model cannot accommodate data of this form. The percentages of mammary tumors in the different female groups was not statistically elevated, but can be used

in GLOBAL83 to calculate an upper-bound risk q_1^* on the hypothesis of a real biological effect. The male rat data, which indicated an elevated mammary tumor response at the high-dose point (not significant) are also used in this manner (Table 32).

For comparison, the combined mammary tumor data from the NTP (1985) study in F344/N rats (using the same dosing schedule) yielded values of $q_1^* = 0.0311 \times 10^{-3}$ for males and $q_1^* = 0.164 \times 10^{-3}$ for females. Thus, while the mammary tumor incidences were not statistically elevated in the Dow (1980) study, the upper bounds on q_1^* that can be derived from the Dow results are in close agreement with the NTP (1985) study.

At a second site, sarcomas in or around the salivary gland in male rats, the Dow (1980) study found statistically positive results (Table 33). The NTP study did not find an elevated sarcoma incidence in or around the salivary gland.

2) The Dow Chemical Company (1982) inhalation study found evidence of an elevated mammary tumor incidence in female rats (the tumor percentage was statistically elevated at 200 ppm compared with controls). The total number of mammary tumors found also showed an increase with dose, as in the Dow (1980) study. GLOBAL83 is applied to the incidence data to determine the maximum linear dose-response component that is compatible with these data (Table 34).

The estimate of q_1^* derived from this study is high compared with that for mammary tumor incidence in female F344/N rats in the NTP (1985) study ($q_1^* = 0.164 \times 10^{-3}$ ppm^{-1}).

3) The National Coffee Association (NCA)(1982a, b) study found evidence of an increased occurrence of liver tumors in female F344 rats given DCM in drinking water. The GLOBAL83 value for q_1^* is shown in Table 35, calculated on the basis of the experimentally applied dose units and also calculated using the same dose units as in the NTP (1985) study. (This second estimate

TABLE 32. RAT MAMMARY TUMORS

Response	Dose[a] Control	500 ppm	1,500 ppm	3,500 ppm	GLOBAL83 q_1^* ppm^{-1}
Females	79/96	81/96	80/95	83/97	0.201×10^{-3}
Males	7/95	3/95	7/95	14/95	0.040×10^{-3}

[a]Dose administered 6 hrs/day, 5 days/wk, for 2 years.

Value for q_1^* based on administered doses and dose time schedule.

SOURCE: Dow Chemical Co., 1980.

TABLE 33. MALE RAT SALIVARY GLAND TUMORS

Dose[a] Control	500 ppm	1,500 ppm	3,500 ppm	GLOBAL83 q_1^* ($\times 10^{-3}$ ppm^{-1})
1/93	0/94	5/91	11/88	0.043

[a]Dose administered 6 hrs/day, 5 days/wk, for 2 years.

Value for q_1^* based on administered dose and dose time schedule.

Note: These data show a statistically significant ($p < 0.001$) test for a linear trend, and the high-dose group response is elevated compared to the controls ($p = 0.002$).

SOURCE: Dow Chemical Co., 1980.

TABLE 34. FEMALE RAT MAMMARY TUMORS

Dose[a]				
Control	50 ppm	200 ppm	500 ppm	q_1^*
52/70	58/70	61/70	55/70	1.22×10^{-3}

[a]Dose administered 6 hrs/day, 5 days/wk, for 2 years.

Value for q_1^* based on administered dose and dose time schedule.

SOURCE: Dow Chemical Co., 1982.

TABLE 35. NEOPLASTIC NODULES OR HEPATOCELLULAR CARCINOMA IN FEMALE F344 RATS

Dose (mg/kg/day)[a]						
Control	5	50	125	250	q_1^* exp. dose units	q_1^* NTP dose units
0/134	1/85	4/83	1/85	6/85	0.470×10^{-3}	0.22×10^{-3}

[a]Dose delivered in drinking water.

Note: The tumor responses in the 50 and 250 mg/kg/day groups were elevated in comparison with controls at the $p < 0.05$ level.

SOURCE: NCA, 1982a, b.

was derived using the ppm to mg/kg/day conversion factors presented in section 4.7. below.) The NTP (1985) report noted that a positive, but marginal, increase in female rat hepatocellular neoplastic nodules or hepatocellular carcinomas was observed in that study (q_1^* approximately 0.03×10^{-3} ppm^{-1}).

4) The NCA (1983) study in mice found evidence of an increased incidence of liver tumors in males. As with rats, a value of q_1^* using the dose units of the NTP (1985) study was derived, and is shown in Table 36. For comparison, the NTP study, which found elevated rates for the same tumors, yields a $q_1^* = 0.195 \times 10^{-3}$ estimate.

In conclusion, the studies discussed in this section provide some evidence for DCM-induced tumors at sites where the NTP (1985) study found tumors; in addition, the Dow (1980) study showed an increase in salivary gland region tumors in male rats. Estimates of q_1^* have been derived for these sites to indicate the maximum linear component of a tumor dose-response that is consistent with study findings. These upper-bound risk estimates are comparable to, or in some cases, larger than, corresponding estimates derived from the NTP (1985) study for the same tumor sites.

4.7. DERIVATION OF HUMAN UNIT RISK ESTIMATES FOR INHALATION OF DCM

The EPA Health Assessment Document for Dichloromethane (U.S. EPA, 1985) developed estimates of unit risks to humans on the basis of the Dow (1980) findings of salivary gland tumors in rats. The same standard CAG assumptions that were used in that document to convert between animal and human doses are also applied here. It is noted that in this assessment, the extrapolation between high and low doses in humans and animals is done in a different order than in the earlier CAG assessment; however, this does not affect the results. Table 37 summarizes the values of q_1^* derived for tumors found in both sexes of rats and mice in the NTP study (data taken from Tables 19 and 20).

TABLE 36. HEPATOCELLULAR ADENOMAS OR CARCINOMAS IN MALE MICE

Dose (mg/kg/day)[a]					q_1^*	q_1^*
Control	60	125	185	250	exp. dose units	NTP dose units
24/125	51/200	30/100	31/99	35/125	0.995×10^{-3}	0.78×10^{-3}

[a]Dose administered in drinking water.

Note: The response at the 125 and 185 mg/kg/day doses were elevated in comparison with controls ($p < 0.05$).

SOURCE: NCA, 1983.

TABLE 37. VALUES OF q_1^* FOR NTP (1985) BIOASSAY USED TO DERIVE HUMAN q_1^* ESTIMATES

Male rat mammary or subcutaneous tumors	$q_1^* = 0.0540 \times 10^{-3}$ (ppm^{-1} exp. protocol)
Female rat mammary tumors	$q_1^* = 0.164 \times 10^{-3}$ (ppm^{-1} exp. protocol)
Male mouse lung or liver adenoma or carcinoma	$q_1^* = 0.429 \times 10^{-3}$ (ppm^{-1} exp. protocol)
Female mouse lung or liver adenoma or carcinoma	$q_1^* = 0.870 \times 10^{-3}$ (ppm^{-1} exp. protocol)

1) These values are first converted to equivalent rodent values for continuous exposure to DCM in units of mg/kg/day. To make this conversion, the inhalation rates for the rodents must first be estimated. This is done using the following formulas (U.S. EPA, 1985):

$$\text{For mice:} \quad I = 0.0345\ (wt/0.025)^{2/3}\ m^3/day$$

$$\text{For rats:} \quad I = 0.105\ (wt/0.113)^{2/3}\ m^3/day$$

Inhalation rates are calculated using the NTP (1985) average weights for male and female mice and rats at the midpoint of the bioassay (51-week data point; the NTP report does not give the average animal weight over the whole study period). The data are given in Table 38.

Dose conversion factors can now be calculated between the NTP schedule and a continuous mg/kg/day exposure. For example, in male rats:

$$1 \text{ ppm NTP schedule} = 10^{-6} \times 3{,}478 \text{ g DCM/m}^3 \times 1{,}000 \text{ mg/g}$$
$$\times \text{ average exposure } 4.29 \text{ hrs/day} \times 1 \text{ day}/24 \text{ hrs} \times 0.268 \text{ m}^3/\text{day}$$
$$/0.462 \text{ kg} = .361 \text{ mg/kg/day}$$

These data are given in Table 39.

2) Equivalent human DCM doses and q_1^* values are now calculated using the CAG methodology for well-absorbed vapors (DCM in air is likely to be absorbed by the lungs to a high degree at low doses in both humans and rodents; the interspecies conversion is being applied for the risks estimated at low doses). The CAG assumes (U.S. EPA, 1985) that humans and animals exposed to equal doses of a carcinogen on a $(mg/kg)^{2/3}$ basis over equivalent proportions

TABLE 38. ESTIMATED INHALATION RATES FOR NTP (1985) TEST ANIMALS

	Weight at bioassay midpoint (kg)	Estimated inhalation rate (m^3/day)
Male rat	0.462	0.268
Female rat	0.278	0.191
Male mouse	0.037	0.0448
Female mouse	0.032	0.0407

TABLE 39. DOSE CONVERSION FACTORS AND EQUIVALENT q_1^* VALUES FOR NTP (1985) STUDY

	mg/kg/day equivalent of 1 ppm exposure, NTP protocol	q_1^{*a} $(mg/kg/day)^{-1}$ for continuous exposure[a]
Male rat	0.361	0.149×10^{-3}
Female rat	0.467	0.383×10^{-3}
Male mouse	0.753	0.570×10^{-3}
Female mouse	0.791	1.10×10^{-3}

[a]these values of q_1^*, which apply to the same tumor types as are listed in Table 31, are obtained by multiplying the q_1^* values in Table 31 by the reciprocals of the values in the first column of this table.

of a lifetime will encounter the same degree of cancer risk.

This implies that a rodent with weight W_R, exposed to a dose of D mg/kg/day and a human exposed to a dose of $D(\frac{WH}{WR})^{-1/3}$ mg/kg/day encounter the same lifetime cancer risks. Table 40 contains human dose equivalents and values for q_1^*.

3) To obtain an estimate of the unit risk for a human inhaling 1 $\mu g/m^3$ of DCM over a lifetime, the standard CAG assumption of a human inhalation rate of 20 m^3/day (U.S. EPA, 1985) is applied. A continuous exposure to 1 $\mu g/m^3$ of DCM is equal to an exposure of

$$1\ \mu g/m^3 \times 10^{-3}\ mg/\mu g \times 20\ m^3/day \times 1/70\ kg = 2.86 \times 10^{-4}\ mg/kg/day$$

Using the value of q_1^* in female mice from Table 34, an upper-limit lifetime cancer risk estimate of 4.1×10^{-6} is estimated for this exposure. Alternatively expressed, $q_1^* = 4.1 \times 10^{-6}\ (\mu g/m^3)^{-1}$. Using the relation that 1 $\mu g/m^3$ DCM is equivalent to 2.88×10^{-4} ppm, $q_1^* = 1.4 \times 10^{-2}\ ppm^{-1}$ (continuous exposure).

4) The CAG's potency index is derived by multiplying q_1^* $(mg/kg/day)^{-1}$ by the molecular weight of the compound (84.9 g/mole for DCM) to obtain q_1^* $(mmol/kg/\ day)^{-1}$. For lung and liver tumors, combined, in female mice, q_1^* $(mmol/kg/\ day)^{-1} = 1.1$. This value is in the fourth quartile of the CAG histogram for the potency index distribution.

4.8. HUMAN UNIT RISK ESTIMATE FOR INGESTION OF DCM

Data both from the NTP inhalation study and from the earlier NCA studies of DCM in drinking water will be considered in connection with a unit risk estimate for DCM ingestion. All of the existing studies have limitations for estimating the risks from ingestion of DCM. DCM is rapidly absorbed and sys-

temically distributed following either inhalation or ingestion exposure; thus an inhalation study is relevant for assessing hazards from ingestion exposure. Nonetheless, exposures via the two routes are likely to lead to differing doses reaching individual organs; in particular, an inhalation study may result in a higher degree of exposure to lung tissue and a lesser exposure to tissues in the digestive system than an ingestion exposure.

For this reason the NTP findings for liver tumors, but not lung tumors, in mice are used for quantitative estimation of risk from DCM ingestion. On the other hand, it should be noted that an analysis based on the NTP study may underestimate risks of liver tumors or other digestive system tumors.

TABLE 40. DOSE CONVERSION FACTORS AND EQUIVALENT q_1^* VALUES FOR RODENTS IN NTP (1985) STUDY AND FOR HUMANS

	Human mg/kg/day equivalent for rodent 1 mg/kg/day exposure	q_1^{*a} $(mg/kg/day)^{-1}$ human
Male rat	0.188	0.793×10^{-3}
Female rat	0.158	2.43×10^{-3}
Male mouse	0.0809	7.05×10^{-3}
Female mouse	0.0770	14.3×10^{-3}

[a]These values of q_1^*, which apply to the same tumor sites as those given in Table 31, are obtained by multiplying the q_1^* values in Table 33 by the reciprocals of the values in the first column of this table.

The NCA (1983) drinking water study in mice yielded suggestive but not conclusive evidence of a treatment-associated increase in hepatocellular carcinomas and/or adenomas in males (U.S. EPA, 1985). This finding can be directly used to make an upper-bound risk estimate from ingestion exposure to DCM. However, the NCA study utilized doses that were well below the maximum tolerated dose (MTD) (U.S. EPA, 1985). Thus, it is possible that elevated tumor incidences would have been found in other tissues if the study had been conducted nearer to the MTD.

a) The unit risk from ingestion exposure is obtained using the q_1^* estimate for liver tumors in female mice (the sex with the higher risk estimate): $q_1^* = 0.160\ (ppm^{-1})$. The female mouse data are used for this calculation because the stronger qualitative and quantitative data for carcinogenicity in the liver were obtained for this sex in the NTP (1985) study. If the NTP findings for male mice were used instead, the unit risk estimate given below would be 20% higher. Using the above dose-conversion procedures, this value leads to an equivalent human estimate of $q_1^* = 2.6 \times 10^{-3}\ (mg/kg/day)^{-1}$.

If a 70-kg human drinks 2 L/day of water containing 1 µg/L DCM, the average daily exposure is

$$1\ \mu g/L \times 10^{-3}\ mg/\mu g \times 2\ L/day \times 1/70\ kg = 2.86 \times 10^{-5}\ mg/kg/day$$

Using the above value for q_1^*, the lifetime incremental cancer risk is estimated at $7.5 \times 10^{-8}\ (\mu g/L)^{-1}$.

b) Using the NCA (1983) study, the value for q_1^* in male mice is $0.995 \times 10^{-3}\ (mg/kg/day)^{-1}$, based on hepatocellular carcinomas and adenomas. The male mouse data from the NCA study are used for this calculation because only the male mice showed evidence for carcinogenicity in the liver in this study, which

was conducted well below the MTD. Following the dose-conversion procedure given earlier in this section, the equivalent human value is 1.23×10^{-2} $(mg/kg/day)^{-1}$. The corresponding UCL unit risk estimate for drinking water is 3.5×10^{-7} $(\mu g/L)^{-1}$.

The unit risks calculated on the basis of the two mouse studies are comparable; therefore, the mean value of 2.1×10^{-7} $(\mu g/L)^{-1}$ is used for the unit risk estimate for drinking water exposure to DCM.

4.9. COMPARISON OF ANIMAL AND HUMAN DATA RELEVANT TO CANCER RISK

The risk prediction for human exposure to DCM can be compared with the observed cancer mortality in a cohort of Eastman Kodak employees exposed to DCM (Friedlander et al., 1978; Hearne and Friedlander, 1981).* Such an analysis was included in U.S. EPA (1984) with reference to the finding of salivary tumors in rats, and a parallel calculation will be shown here. The continuous lifetime equivalent DCM exposure of the Kodak employees was estimated to be between 1.88 and 7.52 ppm, based on a 20-year exposure for the 252 long-term workers. Based on the 95% upper-limit slope factor for the preceding section ($q_1^* = 1.4 \times 10^{-2}$ ppm^{-1} for continuous human exposure), the upper bound on the lifetime cancer risk encountered by these workers is estimated to be between 0.026 and 0.105. For the 252 workers, this would translate to a 95% upper limit of 6.6 to 26.5 excess cancer deaths over a lifetime. However, because the study follow-up period was 17 years and most workers were not observed until death, it is probable that only a fraction of the estimated excess cancer deaths were seen. In the absence of a more rigorous method, it is estimated

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*Ott et al. (1983) also reported an epidemiologic study of DCM workers; however, the limited follow-up in this study prevents any adequate comparison with estimated lifetime cancer risks. In the Ott study, the overall mortality was less than 10% for all subgroups during the study period. See U.S. EPA (1985) for further discussion of this study.

that the fraction of cancer cases that would be observed in the follow-up period is approximated by the overall mortality expected in the follow-up: 65.9/252 deaths, or 26%. Thus, a 95% upper limit of between 1.7 and 6.8 cancer deaths due to DCM exposure would have been predicted for the cohort. Using the statistical methods presented by Beaumont and Breslow (1981), the power of the Friedlander study, with 17.8 expected cancer deaths, to detect an excess of 1.7 deaths from total cancer (with 95% confidence) is 0.06; the power to detect 6.8 cancer deaths is 0.31.

Tumors of several types were found to be elevated in the NTP mouse and rat bioassays. Additionally, it cannot be generally expected that humans and experimental animals will show a carcinogenic response at the same sites when exposed to a chemical which is carcinogenic to both. These factors prevent rigorous comparison of the DCM cancer risk estimated from the NTP study with findings of an epidemiologic study for particular cancer sites. However, as a tentative example of power comparisons that may be made, the ability of the Friedlander study to detect excess lung cancer deaths is calculated.

For this example, an estimate of risk for lung cancer is obtained by applying the observed excess of lung tumors in female mice (the sex with the stronger response) to estimate lung cancer deaths in the Friedlander cohort. Taking the q_1^* value for female mouse lung carcinoma or adenoma from Table 5 (0.579×10^{-3}) and applying the procedure of Section 4.7., the upper-bound unit risk estimate for humans is 9.5×10^{-3} (ppm^{-1}) for continuous exposure. Using this risk value with the same procedure followed above for total cancers leads to the estimate of an upper bound of from 1.2 to 4.7 lung cancers due to DCM exposure; the power of the Friedlander study, in which 4.6 lung cancer deaths were expected, to detect such risk is 0.15 and 0.44, respectively.

The preceding calculations show that the Friedlander study does not have

the power to rule out an overall cancer risk, or in the example presented, a lung cancer risk, that is predicted using the upper-bound slope derived from the NTP study.

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