CARCINOGENIC RISK ASSESSMENT FOR OCCUPATIONAL EXPOSURE TO MONOHALOMETHANES

Final Report

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16. Abstract (Limit: 200 words)

There are four monohalomethanes whose common names are methyl fluoride, methyl chloride, methyl bromide, and methyl iodide. Only the last three compounds are of substantial interest; methyl fluoride has little commercial use and will not be considered further. The monohalomethanes are principally used in industry as methylating agents, although they are also used as fumigants and other applications. In the past, the most important factors considered in designing procedures for the safe handling of these compounds have been their neurotoxicity and other acute effects. Because these compounds are alkylating agents, concern has arisen over their potential carcinogenicity. Several studies have suggested that the monohalomethanes are mutagenic in appropriate test systems, and three studies reported in recent years have suggested that methyl chloride and methyl iodide may be carcinogeneic in experimental animals. This report presents a critical review of these and other studies and assesses the carcinogenic potential of each of the three monohalomethanes. It also presents a quantitative assessment of the carcinogenic risks that might be incurred by workers exposed to these compounds in the workplace under a wide range of exposure conditions.

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I. INTRODUCTION

There are four monohalomethanes whose common names are methyl fluoride, methyl chloride, methyl bromide, and methyl iodide.[1] Only the last three compounds are of substantial interest; methyl fluoride has little commercial use and will not be considered further. The monohalomethanes are principally used in industry as methylating agents, although they are also used as fumigants and other applications. In the past, the most important factors considered in designing procedures for the safe handling of these compounds have been their neurotoxicity and other acute effects (1). Because these compounds are alkylating agents, concern has arisen over their potential carcinogenicity. Several studies have suggested that the monohalomethanes are mutagenic in appropriate test systems, and three studies reported in recent years have suggested that methyl chloride and methyl iodide may be carcinogenic in experimental animals. This report presents a critical review of these and other studies and assesses the carcinogenic potential of each of the three monohalomethanes. It also presents a quantitative assessment of the carcinogenic risks that might be incurred by workers exposed to these compounds in the workplace under a wide range of exposure conditions.

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[1]Although the International Union of Pure and Applied Chemists (IUPAC) has adopted the names fluoromethane, chloromethane, bromomethane, and iodomethane for these compounds, the common names will be used throughout this report because of their general familiarity.

Most of the information available on the toxic effects of monohalomethanes on humans is derived from studies of workers exposed to methyl chloride (1, 2, 3). Most of this information has resulted from acute or subchronic exposures to high concentrations, and no studies to evaluate possible carcinogenic effects of long-term exposure are available. One mortality study of workers employed in plants where brominated chemicals were manufactured suggested a possible association between exposure to methyl bromide and testicular cancer, but this study had a number of limitations and its results are inconclusive (see Appendix A for critical review). Accordingly, the risk assessment presented in this report is based entirely on the results of studies on experimental animals.

A thorough quantitative risk assessment provides a structure for evaluating and integrating the available information on the nature and magnitude of risks posed by these chemicals. The conclusion of the assessment presents the magnitude of the "most-likely" and "worst-case" risks incurred by the exposed population, while focusing on the steps in the derivation that are least understood or least substantiated and so contribute most to the uncertainty of the risk estimates. The available data allow precise assessments of risk to be performed only for methyl chloride. However, evaluation of data on structure-activity and biological activity relationships makes possible some estimation of the order of magnitude of risks posed by methyl bromide and methyl iodide.

A. Physical and Chemical Properties

Chemical and physical constants for the three monohalomethanes of interest are listed in Table I-1. The increasing molecular weights of the compounds correlate with increasing melting points, boiling points, and liquid densities. At room temperature (25°C) and atmospheric pressure both methyl chloride and methyl bromide exist as dense gases, while methyl iodide is a volatile liquid. The bond length between carbon and halogen increases with increasing size of the halogen atom. The homolytic carbon-halogen bond energy decreases with increasing halogen size indicating poorer overlap between carbon and halogen orbitals as the disparity in the size of the orbitals increases. Other properties presented in the table represent potential indicators of biological activity.

Because they are each indicative of properties that may relate to biological activity, as discussed below, both homolytic and heterolytic carbon-halogen bond energies are presented in Table I-1. The homolytic bond energy is the energy required to break a chemical bond symmetrically yielding two free radicals:

$$XCH_3 \longrightarrow X\cdot + \cdot CH_3$$

In this case, the electron pair of the chemical bond is split with one electron remaining associated with each fragment. In certain chemical processes, such as nucleophilic displacements, bonds tend to break asymmetrically with both electrons from the

TABLE I-1

CHEMICAL AND PHYSICAL CONSTANTS OF THE MONOHALOMETHANES

	CH_3Cl	CH_3Br	CH_3I	Reference
Molecular weight	50.49	94.95	141.95	(4)
Melting point (°C)	-97	-93.66	-66.5	(4)
Boiling point (°C)	-23.7	3.56	42.5	(4)
Vapor pressure (mm Hg at °C)	760 at -24.0	760 at 3.6	400 at 25.3	(4)
Liquid density	0.9159_4^{20}	1.6755_4^{20}	2.279_4^{20}	(4)
Carbon-halogen bond length (Å)	1.782	1.939	2.139	(5)
Heat of formation at 25°C (kcal/mol)	-19.32	-8.4	+3.1	(6)
Homolytic carbon-halogen bond energy (kcal/mol)[a]	82.4	69.1	56.4	(6)
Heterolytic carbon-halogen bond energy (kcal/mol)[b]	226.7	221.0	214.0	(6)
Neutral hydrolysis rate constant at 25°C (sec^{-1})	0.235×10^{-7}	4.069×10^{-7}	0.742×10^{-7}	(7)
Swain-Scott selectivity constant	0.938[c]	1.00[d]	1.09[c]	(8)

[a]Derived from gas phase heats of formation for the products and reactants of the equation: $XCH_3 \longrightarrow X\cdot + \cdot CH_3$ (see text). Heats of formation were obtained from ref (6).

[b]This is a gas phase bond energy derived from heats of formation for the products and reactants of the equation: $XCH_3 \longrightarrow X\!:^- + CH_3^+$ (see text). Heats of formation were obtained from ref (6).

[c]Calculated in ref (8).

[d]This value is defined in ref (9).

bond pair remaining associated with one fragment. The energy required to break a bond asymmetrically is the heterolytic bond energy:

$$XCH_3 \longrightarrow X:^- + CH_3^+$$

In the case depicted, the products are ions. As indicated in Table I-1, heterolytic bond energies for the monohalomethanes appear to decrease monotonically with increasing halogen size in parallel to the homolytic bond energies. This situation is complicated by the effects of solvation, however. Although solvation energies for neutral species tend to be small (on the order of several kcal/mol), solvation energies for ions tend to be large (on the order of tens of kcal/mol) so that apparent heterolytic bond energies in solution may vary significantly from the gas phase values reported in the table. Although the overall effect would be to reduce the bond energies reported, bond energies for each of the monohalomethanes would be reduced by different amounts. In general, solvation energies decrease with increasing ion size so that those for the monohalomethanes would decrease in the order: $Cl^- > Br^- > I^-$(10). Correspondingly, the heterolytic bond energies would be reduced most for methyl chloride, less for methyl bromide and least for methyl iodide. Since this would tend to mitigate the differences apparent in Table I-1, a detailed evaluation would be required to determine the actual trend in heterolytic bond energies among monohalomethanes in solution.

Finding a chemical indicator for the relative biological activity of a series of compounds requires knowledge of the transport and reactivity of such compounds within an organism. The biological activity of a specific compound is the net effect of a complicated combination of processes. Even if the scope of a chemical indicator is limited to a specific target effect (e.g., mutagenesis), several competing and confounding processes must be considered. For example, depending upon the route of exposure, there will be some relationship between environmental concentrations and the rate of uptake of a chemical within an exposed organism. Once the chemical is taken up, a series of passive diffusion processes (and possibly active transport processes) distribute the chemical throughout the body of the organism. Thus, there is a relationship between the concentration of the substance at a target location within the organism and the concentration at the point of exposure. The biological activity (or rate of reaction) at the target location can then be related to the concentration at the target location (and thus, ultimately, to exposure) with knowledge of the mechanism of the chemical reaction involved. These relationships are further complicated, however, by competing degradation and excretion processes occuring at the target location and throughout the organism as a whole. Since detailed knowledge of each of these processes is not in general available, several simplifying assumptions are required before candidate chemical indicators may be identified.

The studies reviewed in this report suggest that the mechanism by which monohalomethanes may induce cancer is through alkylation of specific DNA sites within the cell nucleus. Although the details of this process are not considered here, the mechanism of reaction is believed to be nucleophilic substitution in which the halogen atom is displaced by a nucleophilic substituent of DNA. Assuming for the moment that this final process is the process which determines biological activity, candidate chemical indicators of biological activity via such a pathway include heterolytic bond energies (especially if the process is thermodynamically limited), neutral hydrolysis rate constants, and Swain-Scott selectivity constants.

Rates of chemical reactions are determined primarily by two factors: temperature and the activation energy for the reaction. The activation energy is a characteristic energy that must be added to the reactants for reaction to occur. It is the energy difference between the reactants and some high energy combination of the reactants--a transition state--from which products are spontaneously generated. Thus, activation energies are good indicators of reactivity.

Nucleophilic substitutions generally proceed through one of two general types of transition states (11). In the first case, the bond between carbon and the leaving nucleophile (such as a halogen) is completely broken prior to addition of the substituting nucleophile (such as a portion of a DNA molecule). This mechanism is generally termed unimolecular substitution

because the rate depends only on the concentration of the dissociating species. In this case, the activation energy is simply the solution phase heterolytic bond energy between the carbon and the leaving nucleophile. In the second case, the bond between carbon and the leaving nucleophile is not broken prior to addition of the substituting associating nucleophile, so that a five-coordinated carbon transition state forms. This is termed bimolecular substitution because the rate is a function of the concentrations of both the species undergoing substitution and the attacking nucleophile. In this case, the activation energy is somewhat less than the solution phase heterolytic bond energy between the carbon and the leaving nucleophile and can be determined in a kinetic study. Depending on the degree to which the bond between carbon and the leaving nucleophile is distorted in the transition state, however, the solution phase heterolytic bond energy may still provide an indication of relative reactivity between homologous compounds undergoing bimolecular substitution, at least in a qualitative sense.

Since studies indicate that nucleophilic attack at a primary carbon center generally proceeds via bimolecular substitution in which the bond between carbon and the leaving nucleophile is incompletely broken, and since monohalomethanes contain primary carbon centers, it is expected that these compounds will undergo nucleophilic substitution via the bimolecular pathway (11). Thus, solution phase heterolytic bond energies, while not representing activation energies in nucleophilic

substitutions of the monohalomethanes directly, may still provide a qualitative indication of reactivity. Solution phase heterolytic bond energies may be derived from the gas phase values presented in Table I-1 by adjusting for solvation of the reactants and products, as discussed above.

In the absence of good activation energy data, several emperical relationships may provide useful chemical indicators of biological activity. In general, the relative reactivity of a series of compounds toward one nucleophile provides an indication of their relative behavior toward other nucleophiles. The neutral hydrolysis rate constants for the monohalomethanes are provided in Table I-1. These indicate that the relative reactivity of the monohalomethanes toward the nucleophile water follow the sequence: $CH_3Br > CH_3I > CH_3Cl$. Presumably, reactivity toward biological nucleophiles would follow a similar sequence.

Swain-Scott selectivity constants can be used to relate the reactivity of monohalomethanes toward water with their reactivity toward other neucleophiles in a more quantitative manner (9). Swain-Scott selectivity constants, s, are derived from rate data for a series of nucleophilic substitutions using the equation:

$$s = \log (k_n/k_{H_2O})/n,$$

where k_n and k_{H_2O} are second-order rate constants for reactions of alkylating agents (such as monohalomethanes) with nucleophilic compounds (kH_2O is specifically the hydrolysis rate constant

where the nucleophile is water), and n is a Swain-Scott parameter representing the strength of the nucleophile represented in the rate constant k_n. Swain-Scott selectivity constants for methyl chloride, methyl bromide and methyl iodide are presented in Table I-1. Swain-Scott parameters for nucleophile strength are available in the literature (12). The nucleophilic substituent in DNA whose alkylation is believed to initiate cancer is reported to have a Swain-Scott parameter n of approximately 2 (13) from which reaction rates for each of the monohalomethanes can be derived.

Rate constants for the reactions between each of the monohalomethanes and the DNA nucleophilic substituent were derived from the data in Table I-1 using the equation relating Swain-Scott constants and nucleophilic reaction rates provided above. The neutral hydrolysis rate constants presented in the table were converted to second-order rate constants by dividing by the concentration of water at room temperature--55 moles/liter. Calculated second-order rate constants for the monohalomethanes are:

CH_3Cl	2.8×10^{-9} liters/mole sec
CH_3Br	5.5×10^{-8} liters/mole sec
CH_3I	1.2×10^{-8} liters/mole sec

Based on these values, the relative reactivity of the monohalomethanes is predicted to be:

$$CH_3Cl < CH_3I < CH_3Br$$

Dividing the rate constants for CH_3Cl into the other two rate constants, the expected ratios of reactivity are 1:20:4 for methyl chloride, methyl bromide, and methyl iodide, repectively. These ratios will be used in this report as approximate measures of the likely relative reactivities of the three chemicals in alkylating DNA.

B. Carcinogenic Risk Assessment

Performance of a quantitative risk assessment requires evaluation of the pertinent literature in order to assess whether the chemical is likely to be carcinogenic in humans and also to develop the data necessary for estimating the risk involved from exposure to the compound. This evaluation is presented in Chapters II and III of this report. In Chapter II, the pertinent literature on carcinogenicity, teratogenicity and mutagenicity for the three monohalomethanes is reviewed and evaluated. From this evaluation it was determined whether the available data were sufficient to meet the requirements for risk assessment. Data on metabolism and pharmacokinetics of the monohalomethanes are reviewed in Chapter III. This review was used to indicate whether there was sufficient information on metabolism and pharmacokinetics to draw inferences about the form of dose-response relationships or about scaling factors for extrapolation of estimated risk from animals to man. Chapter IV of this report is a detailed review of the methodology for quantitative risk assessment. It discusses the various mathematical models used and other assumptions

necessary for risk assessment. Based on the data evaluated in Chapters II and III and the discussion of methodologies in Chapter IV, dose-response models and scaling factors are chosen for use in the risk assessment of methyl chloride. Estimates of potential risks posed by methyl bromide and methyl iodide are based on measures of relative biological activity and are presented without formal mathematical modeling. The basis for these choices and the data generated from the models are presented in Chapter V of the report. A discussion of the assumptions used and limitations of the risk assessment is given in Chapter VI.

II. ANALYSIS OF KEY STUDIES ON CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY OF MONOHALOMETHANES

This chapter includes a critical review of 15 papers and reports which provide significant information on the carcinogenicity, genotoxicity, and teratogenicity of monohalomethanes. This review is the basis for a concluding analysis of the carcinogenic potential of methyl chloride, methyl bromide, and methyl iodide, and a summary of pertinent information on dose-response relationships.

A. Carcinogenesis Bioassay of Methyl Chloride

1. Description of Study

The Chemical Industry Institute of Toxicology (CIIT) has reported on a 24-month inhalation study of methyl chloride in rats and mice conducted for CIIT by the Battelle Columbus Laboratories (14). The objective of the study was to evaluate the chronic toxicity and oncogenic effects of methyl chloride in rats following chronic exposure by inhalation. The studies were initiated in February 1978, and the final revised report was submitted on 31 December 1981. Because this is the only full-scale carcinogenesis bioassay conducted according to modern protocols for any of the monohalomethanes, and because it is available only as an unpublished report and not as a peer-reviewed publication, it is reviewed in detail for this report.

a. Methods

Initially, 90-day pilot inhalation studies were performed, and it was found that inhalation of methyl chloride at concentrations of 750 or 1,500 ppm resulted in inhibition of weight gain in rats. The higher concentration, 1,500 ppm, resulted in elevations in serum glutamic-pyruvic transaminase (SGPT) activity among male mice consistent with an increase in hepatic injury. The chronic studies were initiated on 28 June 1978, with 120 rats of each sex at each exposure concentration (50, 225 and 1,000 ppm). Shortly after the exposures of the rats were begun, the mice were started on their test regimen, again using 120 animals per sex per exposure concentration. At predetermined 6-month intervals, staggered to accommodate the different starting dates for rats and mice, 60 rats (10 of each sex from each dose group) and 60 mice (10 of each sex from each dose group) were sacrificed for toxicologic and histopathologic evaluation. All survivors were sacrificed after 24 months of exposure, except in the groups of mice exposed to 1,000 ppm, in which all but four males had died by 21 months, and the few surviving females were killed at 22 months.

The rats used in this study were of the Fischer 344 strain from the Charles River Breeding Laboratories, Portage, Michigan. They were 5 weeks of age when received and were housed five per cage in conventional polycarbonate cages. They were held in isolation and examined by a veterinary technician prior to their release to this study. They were identified with numbered ear tags.

The male and female mice used in the studies were of the B6C3F1 hybrid strain and were supplied by the Charles River Breeding Laboratories, Wilmington, Massachusetts. They were five and one half weeks of age when received, were housed in polycarbonate cages and were kept in quarantine for 7 days in the animal facility prior to randomization into experimental groups. All animals were weighed and subjected to ophthalmic examination during their respective quarantine periods. The rats and mice were fed ad libitum with Purina Chow 5001. Water (presumed to be of municipal source) was supplied by an automatic system. Food consumption was not measured and the food was not analyzed by Battelle for foreign contaminants. Excess food was removed daily.

Prior to initiation of the inhalation studies, animals were randomized using computer-generated group assignment tables. The randomization was on the basis of body weight and individual number. Based on this randomization, mice were assigned to cage compartments in groups of four of the same sex, so that body weight means and variances were similar for every group of four animals. Rats were caged separately. Some errors were made in the sex determination of the mice, so that 10 female mice became pregnant during the study.

Housing of the animals during the quarantine and setup periods utilized polycarbonate cages and water bottles. Following this period and during the inhalation studies animals were housed in compartment cages utilizing an automatic watering

system. Animals were housed in cages on racks located in environmentally controlled rooms. On exposure days, animals were transferred from the holding areas to the inhalation exposure area where the cages were transferred to the exposure chamber. On successive days of exposure, the positions of the animal cages in the racks were rotated systematically. The study noted that during transportation of the animals, there were several errors relating to different exposure groups. On three days in the third month of exposure, mice scheduled to be exposed to 50 ppm were exposed to 1,000 ppm while mice scheduled to be exposed to 1,000 ppm were exposed to 50 ppm. Corrective measures were taken, and these errors are not judged to have seriously compromised the study.

b. Clinical observations and biochemical studies

Clinical observations were performed twice daily; these observations were continued during weekends. Clinical signs were noted as follows: animal behavior, anorexia, abnormal physical condition, as well as mortality. Ophthalmic examinations were performed by direct ophthalmoscopy. Pupillary dilation was done using Mydriacyl placed in the conjunctival sac. These observations were performed by the animal technician and the ophthalmic physician.

Body weights were determined weekly during the first 6-month period and biweekly thereafter. Mice were weighed in groups of four or fewer, and rats were weighed individually using Mettler PL 3000 balances. Positive control measures

were used to ensure the complete recording of weights and replacement of animals within clean cages. Animals were weighed at times when cages were cleaned. During the last 18 months of the study, data on body weights were acquired using an automated system. Body weight data were compiled and analyzed by standard analysis of variance (ANOVA).

Neural function was evaluated before scheduled 18- and 24-month necropsies with evaluations made on posture and gait with special emphasis on ptosis, blepharospasm, exophthalmos, pupillary reflex, palpebral reflex, extensor thrust reflex, crossed extensor reflex, and clutch response.

Hematology determinations were done on blood samples taken from selected animals at each necropsy following a 22-hour fast. During the fast the animals were allowed free access to water. This period included 6 hours of exposure followed by 16 hours in an open metabolism cage. Urine was collected, but exhaled air was not. Hematology parameters analyzed were hemoglobin, hematocrit, white blood count, red blood count, mean cell volume, mean cell hemoglobin concentration, cell count, and differential white cell count.

Clinical chemistry studies were performed on blood samples collected by cardiac puncture from the mice and rats used for hematology determinations. The sera were analyzed for glucose, blood urea nitrogen, alkaline phosphatase, serum glutamic-oxaloacetic transaminase, and serum glutamic-pyruvic transaminase.

Creatinine phosphokinase was analyzed at the 6-month interim necropsy only.

At each interim necropsy and final scheduled necropsy, urinalyses were performed on the same animals that were selected for hematology and clinical chemistry determinations. Room temperature collection of urine was employed in contrast to frozen collection. The following determinations were made: total volume, specific gravity, pH, glucose, ketones, protein, and occult blood in urine sediment.

c. Necropsy and pathological studies

Necropsy procedures were done according to a standardized protocol using a computerized randomization of animals to select the designated number of rats and mice of each sex from each dosage level. Gross pathology examinations were performed on each animal sacrificed at the 6-, 12-, 18-, 21-, 22-, and 24-month periods, as well as for animals that died spontaneously. The numbers of animals sacrificed or dying in these periods are summarized in Tables II-1 and II-2.

Forty-four tissues were routinely examined from each animal necropsied. Histopathologic evaluations were performed on slides from all these tissues for animals in the control and 1000-ppm groups. For the 50- and 225-ppm groups, histopathologic evaluations were performed only on target tissues (testis, epididymis, kidney, liver and lung for rats; liver, kidney, spleen and brain for mice). "Unusual tissue masses" from all groups were also evaluated. Six organs (brain, gonads, liver,

TABLE II-1

NUMBER OF METHYL CHLORIDE-EXPOSED MICE INVOLVED
IN SCHEDULED SACRIFICES AND UNSCHEDULED DEATHS

Exposure (ppm)	Sex	Scheduled Sacrifice					Unscheduled Deaths	Total
		6 months	12 months	18 months	21, 22 months	24 months	0-24 months	
0	Male	10	10	5	0	20	75	120
0	Female	10	10	10	0	57	33	120
50	Male	9	10	5	0	32	62	118
50	Female	11	10	10	0	57	34	122
225	Male	10	10	5	0	30	62	117
225	Female	10	10	10	0	68	25	123
1,000	Male	10	10	7	2	0	91	120
1,000	Female	10	10	8	18	0	73	119

SOURCE: Ref. 14, Table 1

TABLE II-2

NUMBER OF METHYL CHLORIDE-EXPOSED RATS INVOLVED
IN SCHEDULED SACRIFICES AND UNSCHEDULED DEATHS

Exposure (ppm)	Sex	Scheduled Sacrifice				Unscheduled Deaths	Total
		6 months	12 months	18 months	24 months	0-24 months	
0	Male	10	10	20	65	15	120
0	Female	10	10	10	57	23	120
50	Male	10	10	20	67	12	119
50	Female	10	10	20	62	19	121
225	Male	10	10	20	68	12	120
225	Female	10	10	20	57	23	120
1,000	Male	10	10	20	66	14	120
1,000	Female	10	10	20	61	19	120

SOURCE: Ref. 14, Table 2

kidney and lung) were weighed from all animals killed at the scheduled sacrifices, and organ-to-body-weight ratios were calculated. Livers were examined for accumulation of porphyrins using UV fluorescence.

d. Inhalation exposure

The animals in this study were exposed to methyl chloride for 6 hours a day, 5 days a week (excluding holidays) for a period of 104 weeks. Exposures were conducted in Hinners-type chambers which were 5 m^3 in total volume and permitted the introduction of five tiers of animals on open-meshed flooring. Room air was drawn into each chamber by negative pressure, and the incoming air was conditioned as well as cleaned with an electronic precipitator and bacteriostatic solution. The temperature of the incoming air was 72.8° F on average and relative humidity was 51.9%. The gaseous methyl chloride was administered via mixing with the air on the input side of the chambers. Concentration and distribution were measured using a MIRAN infrared analyzer. As noted previously, animal positions in the chambers were randomized from top to bottom and left to right and were incremented by one position each exposure day.

The chambers were operated at slightly negative pressure at a flow rate equivalent to 12 air changes per hour (1 m^3/min). The nominal exposure concentrations of methyl chloride were 50, 225, and 1,000 ppm. Measured mean concentrations of methyl chloride were within 2% of the nominal values, with coefficients

of variation in the range 6-18%. Pure methyl chloride gas was delivered from a pressure tank through precision rotometers into the incoming air stream. Chamber flow was monitored by a pneumatic sensor, and chamber concentration was determined by gas chromatographic analysis of a sample of air drawn from the chamber every 20-24 minutes. Chamber sampling was done with this gas chromatographic system using an automated device and a rotary valve. Permanent records were kept of each analysis of chamber concentration, including day, sampling port, and concentration measured.

Test material was obtained from Matheson Gas Products Co. as high purity gaseous methyl chloride with a purity of 99.97%. The entire amount used was obtained from the same lot of material, which was analyzed by Battelle upon receipt of the first partial shipment. Material balance of methyl chloride flowing through the chambers was determined by difference in cylinder weight at the beginning and end of operation. Considering the chamber air flow rate, 1 m^3 per minute or 60 m^3 per hour, the calculations done by Battelle indicate that 97% of the methyl chloride could be accounted.

Environmental conditions in the chamber, temperature and humidity, were indicated as having overall mean values of 72.8°F with a range of 61-83°F. The mean relative humidity was found to be 51.9% with a range from 35% to 69%. The statement was made that measurements were originally made using separate temperature and hygrometer instruments mounted in each chamber,

but that these became divergent or in disagreement. The causes were cited as instrument deterioration. Thus, the temperature and humidity were not recorded from each chamber but rather from the room air at the point of intake. The study authors calculated that the temperature of the chamber during operation would have increased substantially as a result of the heat produced by the animals within the chamber. They correctly calculated that the majority of the heat transfer occurred through the walls of the chamber and accepted as a design consideration a 4.5°F increase in temperature at the center of the chamber. Their careful determination of temperature profiles throughout the chamber while fully loaded indicates their appreciation for the effect of crowding within the chamber.

The finding of a temperature rise of 8°F at 30 inches below the top pyramid coupled with the observation of an extreme input temperature of 83°F suggests that some animals could have been exposed to temperatures as high as 91°F. While this observation does not represent a substantial technical flaw in conduct of the experiment, the question must be asked whether the variations in ambient temperature might have caused substantial variations in net inhaled dose.

The report stated that measurable quantities of methyl chloride were detected occasionally in the control chamber. That is, the valving system that was utilized for the automatic sample acquisition may have contaminated the control air with residual quantities of methyl chloride from the previous sample

(1,000 ppm). Additionally, other factors such as trapped feces or water in the gas sample line could result in spurious readings and the authors' report indicates this. It was also noted in the report (p. 42) that significant losses (leakage) of gas from the supply line to the chamber room atmosphere occurred on widely separated days. Thus, the report indicates that leakage occurred in the common chamber air. It also indicates that a leak was documented when the high concentration chamber was prematurely opened. Such findings suggest that the controls were not rigorously and totally excluded from exposure to methyl chloride. Persons operating the chamber, i.e., the Battelle Columbus staff, may have had unknown exposure. Appendix B of the report indicates that the long-term average concentration of methyl chloride in the control chambers was 0.3 ppm, with transient peaks up to 50 ppm. As noted previously, the record also documented that a low-dose group (50-ppm mice) received a high dose, while a high-dose group was exposed to a much lower level during a 3-day period. Additionally, mice exposed at 50 and 225 ppm received 30% less exposure than was targeted because of an error in calibration of the gas chromatograph. These errors in exposure of the mice resulted from incorrect calibrations after the study on rats had ended. During the final portion of the exposure period, mice were exposed to more than 1,000 ppm.

e. Statistical analysis

Data on organ weights, body weight, and clinical chemistry were analyzed by the following statistical procedures. The Bartlett test for homogeneity was performed on the data, and if no significant differences were found, a one-way analysis of variance (ANOVA) for equality of dose group means was carried out. Where significant differences were found in the analysis, specific dose levels versus control comparisons were made using Dunnett's test. When Bartlett's test was significant so that Dunnett's test or ANOVA were not applicable, the Kruskal-Wallis test was used in place of the analysis of variance. Comparisons of specific dose levels with controls were made using Dunnett's non-parametric equivalent. Additionally, Chi-square tests were used to determine homogeneity of clinical, ophthalmic, and neuro-behavioral observations in male and female rats and mice.

The mortality and lesion incidence data in rats and mice were analyzed using a life table (actuarial) method. Survival curves were calculated from the data by the method of Kaplan and Meyer. Statistical comparisons between and among groups were performed using a generalization of the Fisher-Irwin test for linear trend and by the Cox/Tarone method which incorporates adjustments for differences in survival.

2. Non-neoplastic Effects

a. Ophthalmic Effects

The following results were obtained from the ophthalmic examination of mice and rats. For mice through the period of the 18-month necropsy, no statistically significant lesions were noted. At 21 and 22 months of age, all 1,000-ppm exposure male and female mice were killed since early mortality had occurred and the remaining animals were debilitated. Cataracts were observed in 1 of 2 males and 8 of 18 females. Among the surviving animals at lower concentrations and at the 24-month sacrifice, 2 of 32 males at 50 ppm, 1 of 57 females at 50 ppm and 1 of 68 at 225 ppm had cataracts. Additionally, and in support of the authors' conclusion that cataracts and corneal opacities were not due to exposure to the chemical, the 24-month necropsy of control animals showed that 1 of 20 males and 2 of 57 females had such opacity. The authors claimed that there were no apparent concentration-effect relationships; thus the lesions were not regarded as compound-related. However, their more frequent occurrence in females compared to males at the 1,000-ppm exposure suggest that cataracts may be a consequence of high level methyl chloride exposure when individuals reach old age.

At 6 months a number of rats had corneal cloudiness or opacity. There was a significant increase in the prevalence of these opacities in rats exposed to methyl chloride as compared

to controls. Corneal lesions observed at 12 months were described as being distinctly different from the corneal cloudiness or opacity that occurred at either 6 or 18 months of age in male or female rats. An apparent intercurrent disease, sialodacryoadenitis, which was diagnosed pathologically at 12 months, could have resulted in changes in lacrimal function. A greater irritant effect of the chemical could then be seen. At 18 months of age, comparable incidences of corneal cloudiness and opacity were reported between control male rats and exposed animals ($p>0.05$). The occurrence of a "prominent anterior lens suture" abnormality was observed in male and female rats, with consistently higher incidence in males.

In their summary of the ophthalmologic effects, the authors of this study indicated that no methyl chloride-related ophthalmic effects were observed in either male or female mice in this study. All other lesions or abnormalities, such as cataract formation and microphthalmus, seen in the mice or rats in this study were considered by the authors to be incidental findings and not related to methyl chloride exposure. It does appear, however, that methyl chloride may produce corneal impairment in male and female rats, at least when a viral disease impairs lacrimal function. The authors stated that "the significance of the two distinct conditions that were observed is unclear."

b. Body weights

As noted previously, the animals were weighed weekly during the first 6 months of the study (actually, during the first 27 weeks for mice, 28 weeks for rats) and biweekly thereafter.

Mean body weights of both sexes of mice and rats exposed to 1,000 ppm were significantly less than those of controls and of animals exposed to lower concentrations. The reduction was greatest in male mice, in which body weights were 15-20 percent lower than those of controls from week 35 onwards. At times during the study, the low and intermediate exposure (50 and 225 ppm) groups had significantly lower mean weights than the controls, but these differences did not remain consistent throughout the exposure.

c. Neural function evaluations

Neural function examinations were performed on animals prior to the scheduled sacrifices at 18 through 24 months. Neural functional impairment (clutch response) that was significantly different from controls was observed in male and female mice at the 1000-ppm exposure level at 18, 21, or 22 months in nearly all animals observed. Animals at the lower concentrations were not affected. By 24 months, 21 percent of the male mice at the 225 ppm exposure level and 43 percent of the males and 22 percent of the female mice at the 50 ppm exposure level had hunched posture. Eighteen percent of the male control mice also had hunched posture.

The authors of the report concluded that this abnormality was not a treatment-related effect, but rather a sign of debilitation due to age. However, they concluded that the weakened extensor thrust/scratch response may have been the result of extended (18-24 month) exposure to 1,000 ppm methyl chloride.

This finding is consistent with pathological findings of lesions in the central nervous system (see below).

d. Hematology, clinical chemistry, and urinalysis

A variety of significantly abnormal findings was noted for male and female mice. Specifically, the presence of abnormal serum enzymes that are consistent with hepatic injury was noted in the high exposure male group. In females, there were significant increases in SGPT at 6 and 12 months in all exposed groups, but these were not associated with specific histopathological changes.

In male and female rats several clinical parameters were altered sufficiently for statistical significance, but the authors concluded that there were no consistent or biologically significant alterations in clinical parameters for either sex.

e. Organ-to-body weight changes

All four groups of animals exposed to 1,000 ppm were found to have decreased body weights at the end of the study compared to the controls. The biological significance of statistically demonstrable changes in organ weights is complicated by this finding. In groups exposed to 1,000 ppm, increased relative heart and kidney weights were observed in female mice and both sexes of rats. Female mice and male rats displayed increased relative liver weights, and decreased brain weights were observed in both sexes of both species. There was no consistent pattern of changes in organ weight in groups exposed to 50 or 225 ppm.

The study found no evidence of porphyria in livers of rats or mice.

f. Survival and mortality

Group analysis of survival data showed significantly reduced survival (relative to controls) only in female mice exposed to 1,000 ppm. Although mortality of male mice exposed to 1,000 ppm was also very high, especially between 19 and 21 months, their survival rates were not significantly lower than those of controls. This was due to unusually heavy early mortality of control males (reaching 20% by 8 months). The primary cause of this early mortality appeared to be fighting for dominance among males housed four to a cage. No significant differences in survival were observed among rats.

g. Clinical observations

The authors suggested that both pathologic and neurologic findings were supported by clinical observations. A finding of decreased hostile behavior in group-housed animals as a result of methyl chloride exposure is suggested. Some animals were missexed or misallocated at the beginning of the study. As a result, 10 females became pregnant during the study, four of them repeatedly. These females were retained in the study.

h. Histopathology findings

As stated above, complete histopathological evaluations were conducted on all animals in the control and 1,000-ppm groups, but only selected target tissues and other grossly observable

lesions were examined in the 50- and 225-ppm groups. Histopathological findings were presented in extensive tables (Tables 5-6 of the Battelle report, 14). Statistical analyses were performed on data for lesions selected for evaluation by a pathologist and a biostatistician; results were presented in Tables 7 and 8 of the Battelle report (14).

In male mice, the principal pathological findings associated with exposure to methyl chloride were in the kidney, liver and central nervous system. In the kidney, tubuloepithelial hyperplasia and karyomegaly were first noted at 12 months in the 1,000-ppm group and increased in severity until most animals in this group were sacrificed or died at 21 months. The overall incidence of these lesions in this group was 52/110, versus 0/110 in controls ($p<0.0001$). Renal cortical tumors were first noticed in this group at 12 months and were significantly elevated in frequency between 18 and 21 months (see next section). Renal cortical cysts were also significantly elevated in this exposure group (8/110, versus 1/114 in controls, $p<0.01$). At the 24-month terminal sacrifice, 1/30 male mice at 225 ppm had a renal cortical cyst and 6/32 at 50 ppm had "microcysts", versus 1/20 control males with a cyst. Interstitial nephritis was significantly more frequent in controls (31/106) than in the 1000-ppm group (7/110, $p<0.001$). This condition in controls was associated with trauma to penile and prepubertal areas and ascending urinary tract infections, and was thought to be secondary to injuries incurred during fighting (p. 138); the lower incidence in exposed animals was associated with

a reduction in traumatic injuries and may have reflected a compound-related reduction in aggressive behavior (p. 74).

In the liver, males exposed to 1,000 ppm had a highly significant incidence of a "toxic hepatic syndrome," including multifocal centrilobular hepatocellular necrosis, karyomegaly, cytomegaly, and vacuolar degeneration. The overall incidence of this syndrome was 90/107 in 1,000-ppm males, versus 2/107 at 225 ppm, 1/109 at 50 ppm, and 1/99 in controls ($p<0.0001$). The frequency of hepatocellular carcinomas was 9/107 at 1,000 ppm, 7/107 at 225 ppm, 4/109 at 50 ppm, and 7/99 in controls. In the actuarial analysis conducted by the Cox/Tarone procedure, the difference in frequency of hepatocellular carcinomas between the 1,000-ppm group and controls was marginally significant ($p=0.0169$) and the dose-related trend was highly significant ($p=0.0042$). The authors commented on this finding in the text of the report (p. 138) but omitted it without explanation from their conclusions (p. 148) and summary (p.v).

Other lesions that were significantly increased in frequency in male mice exposed to 1,000 ppm were degeneration and atrophy of the seminiferous tubules (10/101 versus 1/100, $p=0.001$), degneration and atrophy of the cerebellar granular layer (63/99 versus 0/101, $p<0.0001$), and atrophy of the spleen (24/102 versus 4/103, $p<0.0001$).

In female mice, the only significant pathological findings were the toxic hepatic syndrome (28/105 at 1,000 ppm, 1/119 at 225 ppm, 0/116 at 50 ppm, and 0/107 in controls, $p<0.0001$),

and degeneration and atrophy of the cerebellar granular cell layer (data not tabulated, but stated to be significant at $p<0.05$).

No kidney tumors (other than lymphomas) and no other pathological lesions of the kidney were reported in female mice exposed to 1,000 ppm. No hepatocellular carcinomas were identified in female mice and the incidence of hepatocellular adenomas was low in all groups.

In male rats, there was a significantly higher incidence of testicular tubular degeneration and atrophy in exposed groups than in controls at 6, 12, and 18 months. By 24 months, all rats had either hyperplasia or tumors of the interstitial cells, but these tumors were significantly larger in control than in exposed rats. No other pathological lesions were significantly increased in either male or female rats.

3. Kidney Tumors

As noted above, the most significant pathological finding in this study was an increase in kidney tumors in male mice exposed to 1,000 ppm methyl chloride. These tumors were reported as renal cortical adenomas, renal cortical adenocarcinomas, papillary cystadenomas, tubular cystadenomas, and papillary cystadenocarcinomas. However, these tumors were not illustrated or described, and criteria for their diagnosis were not stated. The first renal tumor was a cortical adenoma in one mouse killed at the 12-month sacrifice. One cortical adenoma and one cortical adenocarcinoma were observed in mice dying at 17 months. Two cortical adenomas were observed in mice killed at the 18-month

sacrifice. The remaining 17 renal neoplasms (8 cortical adenomas, 4 cortical adenocarcinomas, 2 papillary cystadenomas, 1 papillary cystadenocarcinoma, and 2 tubular cystadenomas) were observed in 13 mice dying between 18 and 21 months. Two renal cortical adenomas were observed among the 36 mice in the 225-ppm group at the 24-month sacrifice. No renal tumors were observed in controls or in the 50-ppm group. In addition to the statistical significance of the increased tumor incidence at 1,000 ppm, the authors of the report pointed out that these tumors are rare in control mice and were not seen in controls in this study; hence they concluded that the two tumors in the 225-ppm group were associated with exposure to methyl chloride, although this finding by itself was not statistically significant.

A question that may be significant in risk assessment is whether the tumors were associated with overt tissue damage in the kidneys. If such an association were found, it would be consistent with a hypothesis that methyl chloride might induce kidney tumors by an indirect process involving tissue damage and subsequent cell regeneration. Review of the pathological tables appended to reference 6 shows that 17 of the 20 animals with kidney tumors also had other lesions of the renal cortical tuboepithelium. In most cases these were diagnosed as hypertrophy, focal karyomegaly and/or multifocal karyomegaly; in addition, three animals had cortical cysts, and two or three had focal or multifocal hyperplasia. One animal in the 1000-ppm group which had a cortical adenoma at the 12-month sacrifice

had no other kidney lesions. Of the two animals with cortical adenomas in the 225-ppm group, one had no other kidney lesions and the other had chronic multifocal interstitial nephritis. No animals had kidney necrosis. Thus, although most of the animals with tumors also had other renal cortical lesions that were probably related, none showed signs of necrosis and cell regeneration, only two or three had focal hyperplasia, and three (the earliest animals in their exposure groups to develop tumors) had no other tuboepithelial lesions. These data provide no evidence of an association between the development of tumors and overt tissue damage.

4. Assessment and Conclusions

This study was generally well designed and conformed to present-day protocols. However, two features of the study led to substantial limitations in its usefulness for risk assessment. One was the housing of animals four to a cage, which led to substantial fighting and early mortality among control male mice, and hence in a loss of statistical sensitivity. The other was the selection of exposure concentrations in the ratios 0 : 1 : 4.5 : 20; as discussed later, this wide spacing of dose levels makes the results unsuitable to resolve important questions about the shape of the dose-response relationship. Of lesser significance but a potentially important omission was the failure of Battelle to analyze the animals' diet for possible contaminants: this means that the possible contribution of contaminants such as aflatoxins, lead, or PCBs cannot be

assessed, except as they may have been certified by the supplier of the feed prior to shipment. Although the numbers of animals subjected to scheduled sacrifices (Tables II.1 and II.2) were acceptable by present-day standards, they were much too small to permit sensitive tests for differences in organ weights, clinical parameters or pathological changes.

The study appears to have been conducted adequately, although a number of minor problems were detected and reported. These included: the missexing or misallocation of a few animals at the start of the experiment; the failure to remove missexed animals even when females became pregnant; the sporadic exposure of control animals to low levels of methyl chloride; the exposure of animals to the wrong concentrations of methyl chloride during a three-day period; and the escape of some of the animals from their cages, resulting in losses of an unspecified number of animals. However, in our judgment, all these problems were minor and are not likely to have affected the substantive results of the study.

The selection of exposure concentrations may be subject to some criticism. The results of the 90-day pilot study were not reported, except in a two-sentence summary, so that it is not possible to judge whether the selection of exposure concentrations was reasonable in light of the results. In view of the reported finding of elevated SGPT activity and an increase in cytoplasmic vacuoles in hepatocytes in male mice exposed to 1,500 ppm, the selected concentration of 1,000 ppm

for the high exposure level in the chronic study may have been excessive. It proved to be so, because male mice exposed to 1,000 ppm suffered a 15-20 percent reduction in body weight and heavy mortality between 19 and 21 months. This substantially reduced the sensitivity of the study to detect increases in tumor incidence in male mice, and in fact may have reduced the significance of the increase in liver tumors. It also complicates the analysis of the dose-reponse relationship, as discussed below. The selected concentration of 1,000 ppm as the high exposure level for rats may have been slightly below the maximum tolerated dose, although both sexes of rats exposed to this concentration showed small reductions in body weight.

With two important exceptions, the study was well reported and was documented adequately to support its conclusions. One of the exceptions was the failure to describe the observed neoplasms or to present criteria for the reported diagnoses. As a result of this omission, it is impossible to form an independent opinion of the validity of the pathological findings. In the case of the kidney tumors, this is probably not a major problem, since kidney tumors are unusual in mice and the qualitative finding of carcinogenicity is not dependent upon the criteria used for identifying different types of tumors. However, this may be a significant problem for the liver tumors, in which the criteria for distinguishing between carcinomas and adenomas, for example, are often a matter for controversy.

The other major defect in reporting of the study was the authors' failure to report the significant dose-related increase in liver tumors in male mice in their conclusions or summary.

With the limitations pointed out above, this study appears adequate to define the patterns of chronic toxicity and oncogenicity of inhaled methyl chloride in Fischer rats and B6C3F1 mice. In mice, the principal target organs for methyl chloride toxicity are the liver, the kidney, and the central nervous system. In female mice, significant effects were observed only at 1,000 ppm, consisting of the "toxic hepatic syndrome" and degeneration and atrophy of granular cells in the cerebellum; the latter was associated with deficits in neural function. Male mice were considerably more sensitive to methyl chloride than females. Effects observed at 1,000 ppm included increased mortality, liver, kidney and CNS lesions, and increased incidence of kidney tumors and probably liver tumors. Kidney tumors were induced at 225 ppm, and kidney cysts at 50 ppm. Thus, the experiment did not establish a no-effect-level for non-neoplastic effects of methyl chloride in mice.

Rats appeared to be much less sensitive to methyl chloride than mice. The only effect regarded as significant by the authors of this study was degeneration and atrophy of seminiferous tubules in male rats exposed to 1,000 ppm. No significant histopathological effects were observed in any exposed group of female rats. However, in our judgment, weight should be placed on the observed findings of increased corneal opacity

in both sexes of rats exposed to all three concentrations of methyl chloride. This indicated that methyl chloride has the potential to cause adverse effects at relatively low concentrations, at least in conjunction with a viral disease which compromises lacrimal function.

The most significant finding of this study for the purposes of carcinogenic risk assessment is the highly significant increase in incidence of kidney tumors in male mice. In view of the rarity of kidney tumors in control mice, this finding provides sufficient evidence of the carcinogenicity of inhaled methyl chloride in male mice. The study also showed a significant dose-related increase in liver tumors in male mice when data were analyzed by an actuarial method. However, in view of the lack of histopathological criteria for this relatively small effect, this finding needs further investigation, preferably by independent pathological review of the liver slides. Because the frequency of liver tumors in untreated mice is variable, the results should also be compared with data on historical controls, and the small effect observed may be judged not to be biologically significant.

5. Dose-Response Relationship for Kidney Tumors

Table II-3 summarizes the number of male mice sacrificed or dying in each month of the study from the 12th month onwards. The temporal distribution of deaths varied markedly among the exposure groups, which complicates the analysis of the dose-response relationship. The most rigorous way to allow for the

TABLE II-3

DATES OF DEATH OR SACRIFICE OF MALE MICE
IN THE STUDY BY CIIT (6)

Months	Control	50 ppm	225 ppm	1,000 ppm
13*	12	10	10	11 (1A)
14	1	0	0	3
15	3	0	0	4
16	0	0	0	2
17	2	0	0	3 (1A, 1C)
18	1	0	0	3
19*	6	5	5	11 (3A)
20	3	4	2	17 (4A)
21	6	5	1	24 (4A, 4C)
22	3	0	3	3
23	4	1	0	0
24*	26	36	36 (2A)	1
Total	67	61	57	82

*Including scheduled sacrifice

Parentheses indicate numbers of animals with kidney tumors, classified as adenomas (A) or carcinmas (C).

difference in survival among the exposure groups is to fit the data in Table II-3 to a model of the dose-response relationship that explicitly incorporates the time of appearance of tumors. This is one of the procedures used in Section V of this report. However, to use models of the dose-response relationship that utilize only dichotomous data (animals with or without tumors) it is necessary to censor the data to achieve greater similarity in age-distribution among the exposure groups.

The simplest way to allow for the different frequency of early deaths is to exclude all animals that died before the 13th month, when the first kidney tumor was observed: this procedure leads to the incidence figures in the first line in Table II-4. However, these figures underestimate the lifetime probability of tumors in the 1,000-ppm group, because of the reduced lifetimes of animals in this group. To obtain incidence figures that are more comparable between groups, we excluded all animals killed or dying after the 21st month, yielding the figures in the second line in Table II-4. However, this may over-correct for early deaths in the 1,000-ppm group, because the average age of the animals dying between 12 and 21 months in that group was greater than that in the 225-ppm group. For a more rigorous comparison, we used a random number table to select animals from each exposed group so that the distribution of animals by month was the same in each dose group: this procedure yielded the figures in the third line of Table II-4. Unfortunately, this procedure limited the sample size in each

TABLE II-4

INCIDENCE OF KIDNEY TUMORS IN MALE MICE
UNDER VARIOUS SELECTION RULES

Groups Selected	Control	Exposure Group 50 ppm	225 ppm	1,000 ppm
Benign and malignant tumors combined				
All animals, 12-24 months	0/67	0/61	2/57	18/82*
All animals, 12-21 months	0/34	0/24	0/18	18/78*
Age-matched groups+ 12-21 months	0/18	0/18	0/18	3/18
Malignant tumors only				
All animals, 12-24 months	0/67	0/61	0/57	5/82
All animals 12-21 months	0/34	0/24	0/18	5/78
Age-matched groups+ 12-21 months	0/18	0/18	0/18	0/18

+Matched by random selection; see text

*Animals with multiple tumors

SOURCE: Compiled from data in Ref. 14

group to 18 animals, because of the mismatch in the distribution of deaths between the 225-ppm and 1000-ppm groups (Table II-3). The last three lines in Table II-4 refer to the same censored groups of animals, but list data only for malignant tumors (carcinomas).

An important question in risk assessment is whether the results of this experiment are consistent with a linear dose-response relationship or whether they require a non-linear term in the dose-response function. To address this question, we fitted the six sets of incidence data in Table II-4 to the one-hit model of the dose-response function (see Section IV of this report) and applied a Chi-square test for goodness-of-fit. None of the sets of data deviates significantly from the one-hit model ($p>0.05$). Application of the Weibull model to the data in Table II-3, as described in Section V of this report, yielded a non-linear term in the dose-response function, but the coefficient of the non-linear term was not significantly different from zero. Hence, although most of the tumors were observed in the high-dose group, the existence of a non-linear term in the dose-response relationship cannot be established unequivocally. The reasons for this are the following: (i) the intermediate dose-level was too low in relation to the high dose to provide much statistical information; (ii) the statistical usefulness of the data at the intermediate dose was further reduced because many of the intermediate-dose animals survived longer than any of the high-dose animals.

B. Studies of the Carcinogenic Potential of Methyl Iodide

1. Induction of Injection-Site Sarcomas

Preussman (15) and Druckrey et al. (16) reported a study of the potential of 12 direct-acting alkylating agents to induce local sarcomas after subcutaneous injection in rats. The substances selected for study included 3 alkyl halides, one of which was methyl iodide. The rationale for studying direct-acting alkylating agents was that these compounds had previously been considered to be weak carcinogens or noncarcinogenic, in contrast to indirect-acting alkylating agents (those that are metabolized in the organism to form alkylating agents), many of which were known to be potent carcinogens (15). Preussman (15) presented only preliminary results of this study and this evaluation is limited to the full report by Druckrey et al. (16).

The alkylation reaction is associated with the spontaneous and intermediate formation of a carbonium ion, which reacts with nucleophilic sites such as those found in DNA and proteins. The alkylation potential of each compound was determined utilizing the reaction of the alkylating agent with 4-(4-nitrobenzo)pyridine (NBP). The values for NBP binding had been measured and previously reported by Preussman et al. (17). However, Druckrey et al. noted that carcinogenic potential does not necessarily correlate exactly with binding to NBP. The type of electrophile that occurs in vivo may differ from that which occurs in vitro, or reactivity may be modified by solvent effects or effects related to distribution or premature detoxification.

Methyl iodide was selected for testing because of its high reactivity. Druckrey et al. (16) stated that spontaneous heterolysis occurs in vivo to yield carbonium ions. The LD_{50} by subcutaneous injection was found to be 110 mg/kg (0.78 mM/kg) with a survival time of 1-2 days. Hemorrhagic inflammation occurred at the injection site.

The experimental determinations of carcinogenicity were made using 100-day-old rats (sex unspecified) of the BD strain. Methyl iodide (of unspecified purity) was injected subcutaneously in peanut oil. The authors did not mention any concurrent controls, but stated that peanut oil "in control experiments has never produced local sarcomas at the location of the injection, even at high doses." Dosages of methyl iodide administered were 10 and 20 mg/kg with 16 rats being used in the 10-mg/kg group while only 8 animals were used in the 20-mg/kg group. Weekly subcutaneous injections at unspecified sites were given for 1 year, and the treatment was discontinued when necroses appeared. The total doses given were 500 and 900 mg/kg, respectively. The necroses healed rapidly, and following a "short period," tumors became palpable and grew to considerable size.

Six animals were lost prematurely to pneumonia, and the numbers of animals at risk in the 20-mg/kg and 10-mg/kg groups were 6 and 12, respectively. Of these animals, 6/6 and 11/12, respectively, developed malignant tumors. Fifteen of these were local sarcomas and were characterized as fibrosarcomas (11), fusiform cell sarcomas (3) and round-cell sarcoma (1).

Multiple metastases to lung and lymph nodes were seen, and some tumors grew after transplantation into other rats of the same strain. Two sarcomas at distant sites (a paravertebral osteogenic sarcoma and a differentiated sarcoma of the uterus) as well as a benign hepatoma were seen in the 10-mg/kg/week group. The first local sarcoma was seen at about 450 days and a 50% incidence was reached at about 600 days.

In an extension of this experiment, 14 rats were given a single subcutaneous injection of 50 mg/kg of methyl iodide. The authors did not report whether this single dose produced local necroses. Four of the rats developed local sarcomas between 446 and 654 days after administration; two other rats developed differentiated sarcomas at distant sites (colon and vagina). The authors concluded that "[t]he carcinogenic effect of methyl iodide thus appears to be proven. The effect is, however, weak as measured by the length of the induction period and, in keeping with the high chemical reactivity, essentially locally restricted."

Two other alkyl halides were tested in the same study. Benzyl chloride also produced local sarcomas, but appeared less potent than methyl iodide, in keeping with its lower reactivity with NBP. However, veratryl chloride (3,4-bis-(methoxy) benzyl chloride) did not produce local sarcomas, despite higher reactivity with NBP than benzyl chloride and despite the induction of local necroses. This and results with other groups of chemicals showed no consistent relationship between the

reactivity of chemicals with NBP and their carcinogenic potency in vivo.

This experiment suffers from a number of defects in reporting, from the small size of the experimental groups, and from a lack of concurrent controls. Nevertheless, it appears sufficient to show that methyl iodide induces local sarcomas after subcutaneous injection into BD rats, both after single and repeated dosing. It suggests that methyl iodide may also induce sarcomas at distant sites (in contradiction to the authors' conclusion), but this effect is uncertain in the absence of data on controls. The data on veratryl chloride and other compounds show that the effect is not dependent on the induction of local necrosis. Comparison of the results for 12 alkylating agents shows no consistent relationship between reactivity with NBP in vitro and carcinogenic activity in vivo.

2. Induction of Lung Tumors in Strain A Mice

Poirier et al. (18) reported a study of the carcinogenic potential of 17 alkyl halides and 3 pyrimidine analogs, using the assay for the induction of lung tumors in strain A mice described and assessed by Shimkin et al. (19, 20). The test materials were administered by intraperitoneal injection to groups of 20 male and female mice of the A/He strain, starting at 6-8 weeks of age. Methyl iodide (more than 98% purity) was administered 3 times weekly in a vehicle of tricaprylin for 8 weeks. The total doses administered to 3 groups of mice were reported as 0.31, 0.15, and 0.06 mmoles/kg body weight

(i.e., about 1.8, 0.9, and 0.36 mg/kg per dose, respectively). The other compounds were administered on a similar schedule, but at varying doses and in some cases for shorter periods. Groups of 160 and 30 animals served as vehicle and untreated controls, respectively, and two groups of 20 animals were given intraperitoneal injections of urethane (5 and 20 ng/mouse) as positive controls. Surviving animals were killed 24 weeks after the first injection and were examined for pulmonary tumors. "Some" of the tumors were taken for histological examination, but the results were not reported.

Among vehicle control mice, 34/154 survivors had lung tumors for an average of 0.22 tumors per mouse. Among the mice given the highest dose of methyl iodide, 5/11 had lung tumors for an average of 0.55 tumors per mouse. The authors stated that this incidence was significantly greater than that in controls ($p<0.05$). However, they used Student's t-test for this comparison, which is inappropriate since all but one of the mice had either 0 or 1 tumor. Even if the t-test had been appropriate, their numerical calculation appears to have been in error, since recalculation yields a t-value of 1.61 (one-tailed $p>0.05$). Applying a one-tailed Fisher exact probability test, the difference between the proportions of treated and control mice with tumors is not statistically significant ($p=0.104$). The incidences of lung tumors in the middle-and low-dose mice were also not significantly different from those in controls (6/20 and 4/19, respectively, $p>0.1$).

This experiment was conducted in conformity to the accepted protocols for this type of assay (19, 20). However, because of the small numbers of treated animals and the early mortality of nearly half of the animals in the high-dose group, the experiment was too insensitive to give statistically convincing evidence of carcinogenicity of methyl iodide. The authors' conclusion that methyl iodide was the most active of the 17 alkyl halides tested, and was comparable in activity to urethane, is not acceptable in view of the fact that the activity of methyl iodide did not differ significantly from zero. The experiment gave convincing evidence of induction of pulmonary tumors by several other alkyl halides (n-propyl iodide, sec-butyl chloride, iso-butyl bromide, sec-butyl bromide, and n-butyl iodide) but these chemicals were less toxic and could be administered at much higher doses (13-65 mmole/kg).

C. Studies of the Potential Teratogenicity of Methyl Chloride and Methyl Bromide

1. Inhalation Studies with Methyl Chloride in Rats and Mice

The Chemical Industry Institute of Toxicology (CIIT) reported the results of studies of the potential teratogenic effects of inhaled methyl chloride in rats and mice (21, 22).

In the first experiment, groups of 200 male 11-week-old and 200 female 10-week-old CDF Fischer-344 rats were obtained and quarantined for 11 days. The males were caged individually and the females 5 per cage. Monitoring for rat viruses in the home cage rooms was conducted at the beginning and end

of quarantine and at the time of sacrifice, with negative results except for a single titer of 1:160 for murine pneumonia virus. Each female was introduced singly into the cage of a male, and the paper beneath the cage was examined each morning until a copulation plug was observed; this was considered day 0 of gestation. Mating was continued until 100 females with copulation plugs had been collected and randomly assigned to one of four treatment groups. These females were caged individually. On days 7 through 19 of gestation, the caged females were transferred to exposure chambers, in which their positions were randomly rotated daily. They were exposed for 6 hours on each of these days to concentrations of 0, 100, 500, or 1,500 ppm methyl chloride (greater than 99.99% purity) generated from cylinders with an air flow of 250 liters/minute. The females were weighed, and their food and water consumption was measured on days 0, 7, 15, and 20 of gestation.

On day 20, the females were sacrificed. Their uteri were weighed, and the numbers of corpora lutea, implantations, and live and dead fetuses were determined. The fetuses were sexed, weighed, and measured crown-to-rump. From each litter, 50% of the fetuses were stained for skeletal examination and 50% were preserved for soft tissue examination of the head by a modification of Wilson's technique and the trunk by a modification of Staples' method.

The rats in this study and the mice in the study to be discussed next were exposed in the same inhalation chambers.

The realized concentrations for the two experiments are presented in Table II-5. The measured concentrations were close to the nominal concentrations and quite stable. The mean for the middle concentration, which fell 7% short of the nominal level, was least well controlled.

At the time of sacrifice, 22, 23, 20, and 21 of the 25 females in the 0, 100, 500, and 1,500 ppm groups, respectively, were found to be pregnant. The following results pertain to them and their litters. The critical level for statistical significance in all analyses (adjusted for multiple comparisons) was $p<0.05$. The weight gain of the 1,500-ppm females was significantly less than the other three groups on days 7 through 15 of gestation, and in this period the 500-ppm females also showed a significant, but transient, depression in weight gain with respect to the control and 100-ppm groups. Throughout the exposure period, the food consumption of the 1,500-ppm females was significantly less than that of the rats in the other three groups, and, at sacrifice, their average body weight after removal of the gravid uterus was also significantly lower (212.5 g versus 231.0 to 234.7 g). There were no other overt signs of maternal toxicity. The experimental females were not subjected to necropsy. There were no significant differences among the treatment groups in number of corpora lutea, implants, resorptions, dead fetuses, or live fetuses per litter. The only possible suggestion of an embryotoxic effect was in the percent of implantations resorbed: 4.0 ± 1.1, 2.7 ± 1.0, 9.9 ± 5.4,

TABLE II-5

REALIZED CONCENTRATIONS OF METHYL CHLORIDE
IN RAT AND MOUSE TERATOLOGY STUDIES (ppm)

Nominal Concentrations	Minimum	Maximum	Time-Weighted Average ± SD	Percent of Nominal ± SD
100	98	105	102 ± 2	102 ± 2
500	458	497	479 ± 9	93 ± 2
1,500	1,445	1,516	1,492 ± 23	99 ± 2

SOURCE: CIIT (21)

and 6.1 + 4.4% in the control, 100-ppm, 500-ppm, and 1,500-ppm groups, respectively (mean percent/litter ± s.e.). However, these differences were not statistically significant.

Both the male and female fetuses in the high-exposure group weighed significantly less than the fetuses in the other three groups. The 1,500-ppm female fetuses also had significantly shorter crown-to-rump length than the control and 100-ppm fetuses. The high-dose male fetuses showed a similar, nonsignificant reduction in crown-to-rump length. The abnormal findings in the visceral examination were few and unremarkable. The only abnormalities revealed by the skeletal examination were delays in maturation in the high-dose group involving the metacarpals and phalanges of the anterior limbs, the thoracic vertebrae, and the sternebrae. Nonsignificant increases in delayed

ossification of the pubes of the pelvic girdle and the metatarsals of the hind limbs were seen in the 500- and 1,500-ppm groups. The report mentioned a possible dose-dependent decrease in the number of caudal bones and ossification sites, but the data are not compelling.

In summary, exposure of pregnant Fischer-344 rats to airborne concentrations of 100, 500, or 1,500 ppm chloromethane on days 7 through 19 of gestation resulted in fetotoxicity as manifested by retarded growth and ossification only at the highest concentration, which also caused reduction in maternal weight gain. There was no indication of a teratogenic response in any treatment group. The experiment appears to have been designed and conducted satisfactorily, although the sensitivity of the experiment was limited by the small sample size.

The CIIT study of mice (21) exposed to methyl chloride followed a similar protocol to that for the exposure of rats. Groups of 200 C57BL6 8-week-old female mice and 200 C3H 7-week-old male mice were obtained, housed five and one to a cage, respectively, and quarantined for 13 days. Monitoring for mouse viruses at the beginning and end of the study gave completely negative results. The females were then weighed and caged one-to-one with the males. Each morning the vaginal tracts of the females were checked for copulation plugs, and the day on which one was found was designated day 0 of gestation. After 132 females had been found with plugs and 33 were randomly allocated to each treatment group, mating was stopped. These females were housed three to a cage and left undisturbed until

day 6 of gestation. From day 6 to day 17 of gestation, the caged females were transferred into the inhalation chambers for 6-hour daily exposures to nominal concentrations of 0, 100, 500, or 1,500 ppm methyl chloride. Measured concentrations are given in Table II-5. The positions of the cages were rotated randomly within the chambers from day to day. The mice were weighed and their food and water consumption measured on days 0, 6, 14, and 18 of gestation. On day 18 of gestation, the mice were sacrificed and the data collection procedures described previously for the rats were followed.

Exposure of the 1,500-ppm animals was terminated after 4 to 8 days because of progressively more severe manifestations of maternal toxicity. The symptoms included vaginal bleeding, exudate at the eyes, hunched posture, tremors, disheveled fur, bloody urine, and increasingly greater difficulty in righting themselves. The females were weighed and sacrificed at this time, and tissues were taken from the uterus, lungs, kidneys, liver, and brain. It was noted that there was selective necrosis of neurons in the internal granular cell layer. These changes were seen in mice exposed for only 4 days to 1,500 ppm chloromethane.

In the groups of 33 treated females, 24, 20, 17, and 15 were found to be pregnant in the 0-, 100-, 500-, and 1,500-ppm groups, respectively. The results for reproductive and fetal end points are incomplete for the 1,500-ppm group, because of the toxicity and early sacrifice reported above. For the

100- and 500-ppm pregnant mice, there were no significant differences from the controls in weight gain, although the 500-ppm females consumed significantly more food (days 6-14) and water (days 6-18) than the controls. No significant differences were found in the number of corpora lutea, implantations, resorptions, dead fetuses, or live fetuses per litter. The B6C3F1 male and female fetuses in both the 100- and 500-ppm groups tended to be longer and heavier than their control counterparts, but only 100-ppm males showed a statistically significant increase in length over the controls. The skeletal examinations revealed a tendency for more advanced ossification of the fetuses in the treated groups than that seen in the control group. The percent of fetuses affected per control litter was significantly increased only for nonossification of the tarsals, while the fifth and sixth sternebrae and the posterior metatarsals and phalanges showed a similar pattern.

Of most consequence was the finding of nine fetuses in six of the 500-ppm litters with heart defects, involving a reduction or absence of the atrioventricular valves, chordae tendineae and papillary muscles. The defect was seen to affect either the left side (three fetuses) or right side (six fetuses) of the heart, and both male and female fetuses were affected. No heart anomalies were seen in the fetuses of the control or 100-ppm litters.

The authors reported that the difference between the frequency of heart anomalies in the 500-ppm and control litters

was statistically significant using a two-tailed t-test ($p<0.05$). Although the statistical procedure was not clearly stated, they apparently calculated the percentage of abnormal fetuses in each litter and compared the mean percentages between the 500-ppm and control litters. This procedure would be appropriate, but insufficient data were given in the report to verify that this procedure was used or to recalculate the results. Reanalysis of the data using a one-tailed Fisher exact probability test (corrected for multiple comparisons) for differences between the proportions of litters with anomalies yields a significance level of $p=0.0103$.

To confirm this finding of a characteristic heart defect produced in the offspring of mice inhaling 500-ppm chloromethane on days 6 through 17 of gestation, Wolkowski-Tyl et al. (23) undertook another study using concentrations of 0, 250, 500, and 750 ppm methyl chloride. The same protocol was followed with the exception that the group sizes were increased to 74-77 females with copulation plugs. After 7 days of exposure, the 750-ppm females began to exhibit signs of toxicity similar to those seen in the 1,500 ppm animals in the previous study. At sacrifice on day 18 of gestation, the females in the other two treated groups showed no signs of toxicity. None of the treatment groups showed significant increases in fetal loss or impairment of fetal growth. There was, however, a significant increase in the incidence of resorbed, dead, or malformed fetuses in the 750-ppm litters. The previously observed type of heart

defect was observed at significantly increased rates among the 500- and 750-ppm fetuses. A total of 37 affected fetuses was observed, but their distribution over the groups was not specified in the abstract.

These studies on inhalation exposure of pregnant rats and mice appear to have been well conducted and thoroughly documented. They show convincingly that characteristic defects of the heart are produced in B6C3F1 mouse fetuses exposed in utero to concentrations of methyl chloride (500 ppm, 6 hours/day, gestation days 6 to 17) that were not overtly toxic to the dams. Before the conclusion is drawn that methyl chloride is not teratogenic in the Fischer-344 rat, it is to be noted that on a mg/kg/day basis, the exposures of the rats were on the whole considerably lower than those of the mice (see Table II-6), and that the rat experiment involved small group sizes. It remains possible that, if rats were tested more extensively at concentrations of methyl chloride in the range of 750 to 2,000 ppm, some teratogenic potential in this species might also be revealed.

A minor limitation of the experiment is that the dams were not necropsied, so that assessment of the degree of maternal toxicity was based on external observation and on measurements of body weight gain. Thus, it is not certain that the mice would not have shown signs of maternal toxicity if they had been examined pathologically.

TABLE II-6

OBSERVED EFFECTS ON RATS AND MICE
OF AIRBORNE CONCENTRATIONS OF METHYL CHLORIDE
AND ESTIMATED DOSES RECEIVED

Species	Nominal Concentration (ppm)	Estimated* Dose (mg/kg/day)	Maternal Toxicity	Teratogenicity and Fetotoxicity
Mouse	1,500	2,676	very severe (CNS)	?
Mouse	750	1,338	severe (CNS)	heart defect
Rat	1,500	976	mild	retarded fetal development
Mouse	500	892	-	heart defect
Mouse	250	446	-	-
Rat	500	325	-	-
Mouse	100	178	-	-
Rat	100	65	-	-

*Dose conversion from CIIT (21)

2. Inhalation Study with Methyl Bromide in Rabbits and Rats

Sikov et al. (24) reported the results of exposing New Zealand white rabbits to 20 ppm methyl bromide by inhalation for 7 hours each day on days 1 through 24 of gestation or to 70 ppm on days 1 through 15. After 18 days of acclimatization, virgin females were divided into groups of 25 animals balanced for average weight. They were artificially inseminated with pooled semen from three males, and ovulation was induced with intravenous injections of 0.5 ml pituitary luteinizing hormone. The following morning was considered day 1 of gestation.

On days 1 through 24 of gestation, the inseminated females were placed in individual cages within their appropriate exposure chamber and exposed to nominal concentrations of 20 or 70 ppm methyl bromide (99.5% minimum purity) or to filtered air for 7 hours. After the daily exposure period, the females were returned to their individual home cages where food and water were available ad libitum. Because of marked toxicity, the exposure of the high-dose group was terminated on day 15 of gestation. Food consumption was measured every other day, and the rabbits were weighed on days 1, 9, 16, 23, and 30.

The females were sacrificed on day 30 of gestation using CO_2 and necropsied, with any gross internal abnormalities being noted. Lungs, livers, and kidneys were weighed and samples were taken of the ovaries, uterus, liver, lung with trachea, and kidneys and preserved in 10% neutral buffered formalin. For 25% of the animals in each group, these tissues were examined

histologically. For each female, the numbers of corpora lutea, implantation sites, resorptions, dead fetuses, and living fetuses were determined. The amniotic fluid was examined for abnormal color, and the placentas were weighed and examined, with any abnormal ones being preserved for histological examination. The living and recently dead fetuses were weighed, measured crown-to-rump, sexed, and examined under an illuminated magnifier for gross external abnormalities. All fetuses were examined for abnormalities of the head by Wilson's method of serial thin razor blade sections, for internal abnormalities by Staples' modification of Wilson's method, and for skeletal abnormalities by the Staples and Schnell modification of Dawson's method. Statistical analysis of the various types of data were appropriate.

Some difficulties with fluctuations in gas generation and maintaining the integrity of valves in the generation system were reported. Monitoring of the system during each day's exposure period, however, showed that time-weighted average concentrations of 19.3 $\pm$ 0.19 (SD) ppm and 68.7 $\pm$ 2.18 ppm methyl bromide had been realized.

After a week of exposure, the rabbits in the high-dose group began to manifest a pattern of severe toxicity: weight loss, distressed behavior, depressed food consumption, convulsive movements, hind-limb paresis, and ultimately death. Nine had died before treatment was stopped after the fifteenth exposure, and only one of the original 25 animals in this group survived until the scheduled sacrifice on day 30 of gestation. Therefore,

the high-dose group did not provide usable data on the fetotoxic potential of methyl bromide. Such signs of maternal toxicity were not reported for the females exposed to 20 ppm, and there were no deaths in this group or among the controls. The weights of the low-dose and control females did not differ significantly throughout gestation, although after 2 weeks of exposure the treated animals were not gaining weight at the same rate as the controls. On day 9 the average weights in both groups were 3.62 kg, whereas by day 30 they had diverged to 3.77 $\pm$ 0.41(SD) kg for the treated versus 3.95 $\pm$ 0.32 kg for the controls. The patterns of food consumption by the two groups, however, did not differ. There were no remarkable differences between the low-dose and control groups in weight or organ-to-body weight ratios of lung, liver, kidneys, or placenta. Histological examination of the lungs of five animals in each group and the surviving high-dose animal revealed evidence in all of perivasculitis, peribronchitis, and bronchiolitis, accompanied by inflammation and hyperplasia in most. The researchers stated that these lesions were associated with an endemic Pasturella (sic) infection.

At sacrifice on day 30, the numbers of pregnant animals in the control, low-dose, and high-dose groups were 17, 13, and 1, respectively; 13 of the 25 high-dose females had been impregnated. The numbers of corpora lutea and implantations were similar for the control and 20-ppm females. There were no significant differences in the frequencies of resorptions,

dead fetuses, or live fetuses. It is interesting to note, however, that there were 2 dead fetuses in the 13 low-dose litters and none in the 17 control litters. Also, 17 of the 104 implantation sites were resorbed in the 20-ppm litters; two completely resorbed litters, one of 12 implantations and another of a single implantation, account for most of these. The resorption data presented for the controls are discrepant, indicating 11, 12, or 13 resorptions among the 141 implantations depending on whether one looks at number of resorptions, resorptions/litter, or resorptions as a percentage of all implantations. The weight, length, and sex ratio of the fetuses was unaffected by exposure to 20 ppm methyl bromide. The fetuses in the single surviving high-dose litter did show impaired growth. There was no indication of increased visceral or skeletal abnormalities among the 20-ppm fetuses.

Sikov et al. (24) also conducted an investigation of the effect on rats of inhalation exposure to methyl bromide before and/or during gestation. A group of 380 female, 4- to 5-week-old Wistar rats was acclimatized in the laboratory for 10 days before the commencement of the study. They were not tested for infection by Corynebacterium kutcheri, which afflicted rats in the butylene oxide and styrene oxide experiments run at approximately the same time. The researchers assumed that these animals were infected, although they did not develop gross lesions. The females were housed individually in stainless steel cages in one of three inhalation chambers in which filtered

air or air containing 20 or 70 ppm methyl bromide were circulated at a rate of 10 ft^3/minute, giving seven air changes per hour. The animals were assigned by a randomization scheme to give groups of 159, 106, and 106 females balanced for body weight in the air, low-dose, and high-dose groups, respectively. This pregestational treatment was administered 7 hours/day, 5 days/week for 3 weeks. Three days after the last pregestational exposure, the females were mated 2 to 1 with unexposed, 8-week-old male Wistar rats. Vaginal lavages were performed in the morning, and females found to be sperm-positive were randomly assigned to a gestational exposure group with the day of sperm detection being considered day 1 of gestation. These assignments resulted in seven treatment groups with the following combinations of pregestational and gestational exposure: air-air, air-low, air-high, low-air, low-low, high-air, and high-high. Mating and the commencement of gestational exposure continued over 7 to 9 days "until about 36 sperm-positive rats were assigned to each experimental group;" realized group sizes were 38 to 42. The exposures at the same nominal concentrations as in the pregestational period were administered 7 hours/day, 7 days/week on days 1 through 19 of gestation. Throughout the exposure and mating periods, the females were monitored for survival, appearance, and behavior. Their food consumption was measured three times a week during the pregestational exposures and at 2-day intervals during gestation. They were weighed twice each week in the pregestational period and on days 1, 7, 14,

and 21 of gestation. The animals were coded and sacrificed randomly by treatment on day 21 of gestation so that the teratological examination was performed blind. The females and their litters were examined in the same fashion as the rabbits described above with the exception that the rat fetuses were randomly divided into two groups, one to be examined only for skeletal abnormalities and another to be examined for head and internal abnormalities as well as skeletal abnormalities (the latter procedure given preference in litters of four or less). The data were subjected to the same type of analyses as those from the rabbit study.

The realized time-weighted average concentrations of methyl bromide were 19.6 $\pm$ 0.9(SD) ppm and 68.5 $\pm$ 1.7 ppm during the pregestational period and 20.0 $\pm$ 1.5 ppm and 68.8 $\pm$ 1.9 ppm during the gestational period for the low- and high-dose groups, respectively. The only mortality observed during the experiment was the deaths of two high-dose females on the first night of mating, apparently caused by fighting due to their unfamiliarity with multiple caging. The randomization according to weight had been performed "several days before the initiation of exposure" and by the time of the first pregestational treatment, the high-dose females weighed significantly more than the controls (179 g versus 171 g); by the 8th day of exposure, the low-dose females also weighed significantly more than the controls (201 g versus 194 g). The differences remained significant until the 17th day of exposure, when the average weight

in all three groups was exactly 220 g. During days 1 to 14 of gestation, the high-high females weighed significantly less than the unexposed controls, as did the air-high females on day 14. None of the other treatment combinations showed significant differences in weight gain from the controls, but the averages for the high-air group appeared somewhat depressed on days 7 and 14 of gestation. Food consumption was equivalent among all groups for both exposure periods. The tissues of eight females in each exposure combination group were examined histologically. Exposure to methyl bromide did not affect the weight of maternal liver, lung, kidney, or placenta or the organ-to-body weight ratios. Lung lesions that were associated with C. kutcheri infection or some unidentified prior exposure were seen in all groups. Foci of hepatic necrosis were observed in almost all rats from each of the groups. The distribution of five instances of hydronephrosis was more suggestive of a substance-related effect (1 low-air, 1 high-air, 2 air-high, and 1 high-high).

There was no treatment effect on the proportion of sperm-positive females who turned out to be pregnant (87-100% in the seven groups). Comparison of Tables 2 and 17 from the report leaves four exposed (sperm-positive) females unaccounted for; furthermore, the number of litters reported in Table 24 of the report does not correspond to the number of pregnancies recorded in Table 17 of the report. (See summary in Table II-7.)

TABLE II-7

NUMBER OF RATS EXPOSED AND REPORTED
IN STUDY OF SIKOV ET AL. (24)

	Number Reported by Exposure Group						
	A-A	A-L	A-H	L-A	L-L	H-A	H-H
Number Exposed (Table 2) should equal	42 *	40 *	40	38	40	39 *	40
Number Sperm-positive (Table 17)	41	39	40	38	40	37	40
Number Pregnant (Table 17) should equal	40 *	34 *	39 *	35 *	40 *	37 *	38 *
Number of Litters (Table 24)	37	31	36	34	38	36	36

There were no significant differences among the groups in any of the variables measuring reproductive performance. In fact, that of the air-air group was the poorest with 5.3% of implantations resorbed, one of the two dead fetuses in the study, and 11.5 live pups per litter versus 12.2 to 12.9 in each of the six treatment groups. No differences were found in fetal weight, length, or sex ratio. A variety of soft tissue anomalies were observed in control and treated fetuses, but with at most two instances of a particular abnormality seen in a group, there was no indication of a treatment-related effect. Extra and rudimentary ribs were found in all groups

at rates that did not differ among them, as were delayed ossifications of the various bones of the head.

This study appears to give considerable evidence that inhalation of 20 or 70 ppm methyl bromide during pregnancy does not cause fetotoxic or teratogenic effects in Wistar rats. The experimental design does not, however, provide a valid test of the effects of methyl bromide exposure on mating; filling the treatment groups for the gestational portion of the experiment according to a truncation rule ("until about 36 sperm-positive rats were assigned to each experimental group") means that exposed females whose mating behavior had been impaired would have been selectively omitted from the study. About 25% of each pregestational treatment group was discarded in this fashion without any further consideration. Assuming that mating behavior and ultimate reproductive success may be to some extent correlated, this "screening" could in fact have eliminated the more succeptible dams from inclusion (perhaps making the remaining treated females actually more successful than the unculled controls). Each female should have been assigned to a gestational exposure group and followed through this period, or at least very careful account should have been made of the rate at which the females in the different treatment groups became sperm-positive and of necropsy results on those that did not, especially for their reproductive organs. The discrepancies in the numbers upon which the data analysis are

based also detract from the confidence that can be put in these data. The endemic infection may also be indicative of problems with the research facility, although the investigators claimed that the animal supplier was responsible.

Despite these limitations, the experiment provides substantial evidence for the absence of fetotoxic or teratogenic effects in Wistar rats exposed to 20 or to 70 ppm methyl bromide or to New Zealand rabbits exposed to 20 ppm. The usefulness of the study with rabbits is limited by the fact that the higher dose was too toxic to the females to permit evaluation. The selection of 70 ppm as the high dose was inappropriate in view of the cited results of an earlier study, in which exposure of rabbits for 6 months to concentrations of 33 and 100 ppm had caused pulmonary and neurotoxic effects.

D. Studies of Mutagenic and Other Genotoxic Effects of Monohalomethanes

1. Studies Utilizing the Ames Assay for Point Mutations in S. Typhimurium

Simmon (25) reported on studies of the mutagenic activity of a number of alkyl halides, including methyl chloride, methyl bromide, and methyl iodide, in S. typhimurium (strains TA 1535 and TA 100) using a modification of the Ames assay. The published paper primarily addressed structure-activity correlations among the alkyl halides, and the results for the monohalomethanes were reported extremely briefly. The same data were reported with slightly more detail by Simmon et al. (26), and the two papers are reviewed together here.

Although the studies reported by Simmon et al. (26) included five strains of S. typhimurium (TA 1535, TA 1537, TA 1538, TA 98, and TA 100), results for the monohalomethanes were reported only for strain TA 100. This strain is susceptible to base-pair substitution mutations and is more sensitive than other strains used for detecting such mutations (27). The authors reported using the assay procedure described in detail by Ames et al. (27), including the optional use of a metabolic activation system ("S-9 mix") prepared from livers of rats pretreated with Aroclor 1254 or phenobarbital. In this study, agar overlay plates containing bacteria (and, optionally, S-9 mix) were placed uncovered in a 9-liter desiccator. For methyl iodide (a liquid at room temperature), volumes of 10, 20, 30, 40, or 50 µl were added to a glass petri plate suspended beneath the porcelain shelf of the desiccator. For methyl chloride and methyl bromide (gases at room temperature), known volumes of the gas at ambient atmospheric pressure were introduced into evacuated desiccators, which were then equilibrated to ambient atmospheric pressure with filtered air. The authors reported using concentrations of 2.5, 5, 10, 15, and 20% methyl chloride and 0.01, 0.02, 0.04, 0.10, and 0.20% methyl bromide, but did not state precisely how these nominal concentrations were calculated and apparently did not measure the actual concentrations. The reported concentrations were presumably expressed on a volume/volume basis, although this was not explicitly stated. The desiccators contained magnetic stirrers which

served as fans to assure rapid evaporation of the liquids and even distribution of vapors. The desiccators were placed on magnetic stirrers at 37°C for 7-21 hours, and the cultures were then incubated in the absence of the monohalomethane for about 40 more hours at 37°C. Positive and negative (solvent) controls were used in each experiment.

The authors used chemicals "of the highest available purity" available from commercial suppliers. Results for the three monohalomethanes were reported only in graphical form. To the accuracy with which data can be read from the published graphs, the results are tabulated here in Tables II-8 to II-10. The authors stated that at least two plates for each concentration (with or without S-9 mix) were used, and that all experiments were repeated at least once. However, they presented only a single result for each concentration and did not report the variability in their results.

Despite the brevity of each of these two papers, the experiments appear to have followed standard protocols for the Ames assay and to have used an appropriate modification of the assay for the testing of chemicals in vapor form (28). Although the results were not analyzed statistically and variability was not reported, the published data for each chemical show a monotonically increasing dose-response relationship over 4 or 5 concentrations (Tables II-8 to II-10). Thus, these experiments provide sufficient evidence that each of the three monohalomethanes induces base-pair substitution mutations in

TABLE II-8

MUTAGENIC ACTIVITY OF METHYL CHLORIDE
IN THE AMES ASSAY (Ref. 26)

	S. Typhimurium TA 100 Revertants per plate	
Concentration (%)	+S-9 Mix	-S-9 Mix
0	116	83
2.5	530	385
5	824	663
10	1,019	895
15	1,288	1,127
20	-	1,081

TABLE II-9

MUTAGENIC ACTIVITY OF METHYL BROMIDE
IN THE AMES ASSAY (Ref. 26)

Concentration (%)	S. Typhimurium TA 100 Revertants per plate* -S-9 Mix
0	85
0.01	226
0.02	290
0.04	522
0.10	621
0.20	435

*21-hour exposure

TABLE II-10

MUTAGENIC ACTIVITY OF METHYL IODIDE
IN THE AMES ASSAY (Ref. 26)

Concentration (%)*	*S. Typhimurium* TA 100 Revertants per plate -S-9 Mix
0	99
0.04	190
0.09	179
0.18	228
0.22	247
0.31	281

*Concentrations were calculated on the assumption that all liquid methyl iodide evaporated in the 9-liter dessicator.

S. typhimurium when the culture is exposed directly to the vapors. All the chemicals were mutagenic in the absence of metabolic activation with S-9 mix. Methyl chloride was slightly more active in the presence of metabolic activation (Table II-9), but it is not clear that this difference was statistically significant.

The experiment is less satisfactory as a quantitative measure of the biological activity of the three chemicals in this system, for two reasons: first, because the concentrations were not directly measured; and second, because the chemicals were tested at different concentrations and for different times. However, assuming that methyl iodide was fully volatilized and mixed into the 9-liter space, the gaseous concentrations of this chemical would have been as indicated in Table II-10. Assuming that the nominal concentrations were achieved and maintained throughout the period of exposure, the slopes of the dose-response functions (in revertants per percent concentration) were as follows: methyl chloride, 106; methyl bromide, 11,800; methyl iodide, 1320. Since methyl bromide was tested for 21 hours versus 8 hours for the other two chemicals, these data probably exaggerate the relative activity of methyl bromide. Assuming that the number of revertants would be proportional to the duration of exposure (an assumption supported by data presented for four other chemicals in ref. 25), the relative biological activities of the three chemicals in this system would be approximately in the ratios 1:40:10. However, these

ratios are subject to uncertainties, presently unquantifiable, because the assumptions specified above have not been verified.

The fact that all three monohalomethanes yielded positive results in the absence of metabolic activation suggests that they are direct-acting mutagens. This may result from their direct-acting alkylating action. The fact that the mutagenic activity was not changed by inclusion of a metabolic activating system can be explained in several ways: An increase in mutagenic activity was not seen because activated metabolites may not be formed or the metabolites may be equally potent as methyl chloride. A decrease in mutagenic activity was not observed because metabolic detoxification may be slow or does not occur by the metabolic activating system. Additionally, methyl chloride may alkylate the active center of cytochrome P-450, the enzyme responsible for activation or detoxification in the metabolic activating system, and inhibit metabolism of methyl chloride. There is insufficient information to support or discount any of these possibilities.

Andrews et al. (29) compared the mutagenic activity of methyl chloride and vinyl chloride using a modification of the Ames assay. S. typhimurium strain TA 1535 was used in this assay. This strain is sensitive to base-pair substitution mutations, but is less sensitive than TA 100 (27). The bacteria were exposed to the compounds in the presence and absence of a S-9 liver fraction from Aroclor 1254 pretreated animals (species not specified) and necessary cofactors. Both the bacteria

and the metabolic activating system were mixed in molten top agar and spread in petri plates containing Vogel Bonner E medium.

The bacteria were then incubated in an anaerobic jar containing the test gas. The concentration used was obtained by evacuating the jar to a known pressure and then adding test gas, using each incremental increase in pressure by 7.6 mm Hg to approximate 1% of the jar volume. Each test gas used was considered "pure" after gas chromatographic analysis. The concentration of the test gas in the head space of the jar was measured after 24-48 hours by gas chromatography. Five replicate plates were incubated at each concentration. After incubation for 72 hours at 37°C the numbers of revertant colonies were counted.

Both methyl chloride and vinyl chloride were found to be mutagenic in the presence and absence of the metabolic activating systems. The measured atmospheric concentrations of methyl chloride used were 0.5, 0.8, 3.8, 8.7, 13.3, 20.7, and 23%. At a concentration of 23%, methyl chloride was reported to be toxic to the bacteria. All other concentrations used except 0.5% produced a significant increase in the number of revertant colonies (Table II-11). The authors reported that there was no significant difference between the numbers of revertants produced with or without the metabolic activating system. However, at each concentration, the number of revertant colonies was greater when this system was added, and this difference appears significant at concentrations of 0.5 and 0.8% (two-tailed t-test, $p<0.05$).

TABLE II-11

MUTAGENIC ACTIVITY OF METHYL CHLORIDE
IN THE AMES ASSAY (Ref. 29)

Concentration of methyl chloride (%)	*S. typhimurium* TA 1535 revertants per plate	
	S9 absent	S9 added
0	28.9 ± 6.2	14.9 ± 3.7
0.5	31.6 ± 5.6	46.6 ± 9.7*
0.8	53.6 ± 4.0*	79.4 ± 9.7*
3.8	239 ± 44*	269 ± 40*
8.7	928 ± 179*	1,046 ± 144*
13.3	1,600 ± 329*	1,939 ± 559*
20.7	1,558 ± 159*	2,038 ± 416*

*Significantly different from controls ($p<0.01$)

There was an approximately linear dose-response relationship for exposure concentrations of 3.8, 8.7, and 13.3%. The numbers of revertant colonies at the exposure concentration of 20.7% was very similar to the number found at 13.3% both with and without the metabolic activating system, presumably because of the toxic effects noted at the next higher concentration.

This study followed standard protocols and appears to have been well conducted and well reported. The reporting of statistical variability is an improvement over the studies reported in references 25 and 26. This study thus provides sufficient evidence that methyl chloride is mutagenic in S. typhimurium strain TA 1535 with or without metabolic activation. The comments made on the previous study are equally applicable to this one.

Ong et al. (30) reported on mutagenicity testing of 147 industrial chemicals and related compounds, including methyl bromide, in the Ames assay. The tests were reportedly conducted according to standard procedures for the assay (27), using strains TA 1535, TA 1537, TA 98, and TA 100. Chemicals were "obtained from commercial chemical companies" and their purity was not reported. Gases and volatile compounds were "tested in a sealed jar": no other details of exposure procedures were given. S-9 mix was prepared from livers of rats pretreated with Aroclor 1254. The plates were apparently incubated in the presence of the test chemical at 37°C for 3 days, but this was not stated explicitly for gaseous compounds. The text

of the paper stated that each chemical was tested at 5 concentrations and that the criteria for classifying a compound as mutagenic was a dose-related increase in the number of revertants and an increase to more than twice the background level. However, the table of results reported data for only one concentration of each chemical.

For methyl bromide, the authors reported testing at a nominal concentration of 0.53%. This concentration led to a significant increase in revertants only for strains TA 1535 and TA 100. For TA 1535, the numbers of revertant colonies were 124 and 28 with and without metabolic activation, versus 8 and 13, respectively, in controls. The significance of the increase without metabolic activation is questionable, however, because other control plates had up to 43 revertants. For TA 100, the numbers of revertant colonies were 710 and 700 with and without metabolic activation, versus 159 and 151, respectively, in controls.

The reported results of this study are in qualitative agreement with those of ref. 26 in suggesting that methyl bromide causes base-pair substitutions in S. typhimurium strain TA 100. However, the tabulated concentration of 0.53% in this study was greater than the concentration of 0.20% reported to be toxic to the bacteria in ref. 26. In the absence of adequate details about this and other features of this experiment, little weight can be placed on its results.

McCann et al. (31) compiled the results of tests on 300 chemicals for mutagenicity in the Ames assay. The mutagenicity of these chemicals in this system was compared to information on their carcinogenicity or noncarcinogenicity as reported in published studies. The chemicals were grouped into broad classes, although this led to some grouping of dissimilar compounds. Twelve groupings were made and are listed below:

Aromatic amines, etc.
Alkyl halides, etc.
Polycyclic aromatics
Esters, epoxides, carbamates, etc.
Nitro aromatics and helerocycles
Miscellaneous aliphatics and aromatics
Nitrosamines, etc.
Fungal toxins and antibiotics
Mixtures
Miscellaneous heterocycles
Miscellaneous nitrogen compounds
Azo dyes and diazo compounds

Overall, McCann et al. reported a high correlation between carcinogenicity and mutagenicity, with 90% of the carcinogens being mutagenic in the Ames assay (156/174). Although not reported, there was about an equal percentage of the mutagens being carcinogenic. This means that about 10% of the carcinogens and 10% of the mutagens were not shown to be mutagenic or carcinogenic, respectively.

Methyl iodide was placed in the group of "alkyl halides, etc." This group of compounds is listed in Table II-12. Methyl iodide was considered a carcinogen based on a limited study and was stated to be "weakly mutagenic" in S. typhimurium strain TA 100. The reference for the carcinogenicity study was a published compilation (32) and may refer to the study by Druckrey et al. (16) reviewed earlier in this report. The positive result for mutagenicity was not obtained by McCann et al. but was

TABLE II-12

TABULATION BY McCANN ET AL. (31)
OF MUTAGENIC AND CARCINOGENIC PROPERTIES
OF THE "ALKYL HALIDE, ETC." GROUP OF CHEMICALS

Alkyl halides, etc.	Carcinogenicity	Mutagenicity
Methyl iodide	+	W+
Carbon tetrachloride	+	0
Ethylene dibromide	+	W+
Vinyl chloride	+	W+
Vinylidene chloride	?+	W+
Chloroacetic acid	0	0
2-Chlorobutadiene (chloroprene)	?+	+0
bis-(Chloromethyl)ether (BCME)	+	+
bis-(2-Chloroethyl)amine	?	+
Methyl-bis-(2-chloroethyl)amine (nitrogen mustard)	+	+
Uracil mustard	+	+
Cyclophosphamide	+	+
Isophosphamide	+	+
Chlornaphazin	+	+
Melphalan	+	+
2-methoxy-6-chloro-9-(4-bis(2-chloroethyl)amino-1-methylbutyl-amino)-acridine-2HCl (ICR-10)		
2-methoxy-6-chloro-9-(3-(ethyl-2-chloroethyl)amino-propylamino) acridine-2HCl (ICR-170)	+	+

TABLE II-12, Continued

Alkyl halides, etc.	Carcinogenicity	Mutagenicity
2-methoxy-6-chloro-9-(3-(2-chloroethyl) aminopropyl-amino) acridine-2HCl (ICR-191)	?0	+
Dimethylcarbamyl choride	+	W+
Benzyl chloride	?+	W+
Captan	+	+
Folpet	0	+
DDE	+	0
Dieldrin	+	0

+, carcinogen or mutagen

0, not mutagenic or not carcinogenic

?+, carcinogenicity based on limited study

?0, not carcinogenic based on limited study

W+, weakly positive

cited as a personal communication from V. Simmon; it presumably refers to the study reported by Simmon et al. (25, 26) reviewed earlier in this report.

Within the group of "alkyl halides, etc.," 17 of 18 (94%) compounds that were mutagenic were also reported to be carcinogenic, while 17 of 20 (85%) compounds that were reported to be carcinogenic were also mutagenic. However, only 4 of the 23 compounds in the group were listed as not mutagenic and only 3 were listed as not carcinogenic; only one of these was listed as both nonmutagenic and noncarcinogenic (Table II.12). Thus the concordance between findings of carcinogenicity and mutagenicity within this group was reasonably good (85-94%) for positive findings, but poor (25-33%) for negative findings. Also, the group contained a wide variety of compounds, including a number of nonhalogenated compounds and halogenated aromatics (Table II-12).

This paper provided no new evidence about the carcinogenicity or mutagenicity of monohalomethanes. In general, it provided evidence for a correlation between mutagenic and carcinogenic activity over a wide range of chemical substances. However, it provided no substantial evidence for such a correlation within the class of alkyl halides.

2. Study Utilizing the Neurospora Back-Mutation Assay

Kolmark (33) used the Neurospora back-mutation test to investigate the mutagenic activity of some inorganic acid esters, in particular the dialkyl series from dimethyl to dibutyl sulfate.

As part of this investigation, the author also used this test system to examine the mutagenic activity of methyl iodide. The test organism was a mutant strain of Neurospora, K3/17, which has two mutations so that it needs supplemental adenine and inositol for growth. These gene mutations are not linked and thus it is possible to measure the back-mutation rate of both genes independently under the same conditions with identical genetic and physiological backgrounds.

A suspension of multinucleated macrocondia, at a concentration of approximately 25 x 10^6 condria per ml, was exposed to methyl iodide at 25°C for varying time periods and/or at varying concentrations. Exposure ended by washing the condria with sterile water. The condria were then plated on three different media which contained inositol, adenine, or both. These media were used to identify back-mutations at the adenine and inositol loci and to determine cell survival, respectively. Details were not reported on the number of condria plated per plate or the number of replicates, if any, although it was stated that techniques followed those reported in earlier papers (34, 35, 36).

Methyl iodide was tested in this system over a concentration range of 0.01 to 0.1 M, using an exposure period of 30 minutes. This range of concentrations produced survivals ranging from 100% to less than 0.1%. However, no exposure level caused an increased mutation rate at either locus examined.

In the same series of experiments, this system was sensitive to the mutagenic effects of dimethyl sulfate and diethyl sulfate. It was able to detect the mutagenicity of dimethyl sulfate with exposure to a concentration of 0.005 M for 30 minutes. Thus, the assay was not limited by the use of low concentrations or short exposure periods.

3. Study Utilizing the Mouse Lymphoma Cell Assay

Clive et al. (37) reported on an extensive study to validate and characterize the L5178Y/TK^{+}/$^{-}$ mouse lymphoma mutagen assay system. They tested 43 chemicals for which they reported dose-survival-mutagenic response data, including that for methyl iodide, which was used as a positive control. L5178Y Mouse lymphoma cells heterozygous at the thymidine kinase (TK) locus were used in this assay system. These cells, at a concentration of 1 x 10^{6} cells per ml, were incubated in a liquid medium with the test compound for 2 or 4 hours at 37°C. In some assays a metabolic activating system was added to the medium. This S-9 liver fraction was from either normal rats or rats pretreated with Aroclor 1254. Following incubation the cells were washed clean of the test compound and assayed for mutants. The methods used were stated to be those described by Clive and Spector (38), except that trifluorothymidine was at times used instead of 5-bromo-2'-deoxyuridine (BUdR) to select for the TK recessive mutants. In some tests the cells were also assayed for hypo-xanthine-guanine phosphoribosyl transferase (HGPRT) mutants using 6-thioguanine (TG). Following exposure, the cells were allowed to recover in growth medium for periods of 48-168 hours

("expression times") before plating on soft agar with the appropriate selection media. Expression times of 48 or 72 hours for the TK recessive mutant were usually sufficient, while longer expression times were needed for TG-resistant HGPRT mutants. Cell survival was also determined and for a valid assay survival was required to be at least 10%.

Two quantitative measures were used to rank the chemicals. The first was the "mutagenic potency," which was defined as the mutagenic frequency divided by the treatment concentration and the treatment time. The second measure was the lowest effective concentration (LEC), which was defined as the minimum concentration of a chemical required to induce a significant (usually two times background frequency) increase in mutation frequency.

Positive controls included ethyl methanesulfonate which does not need metabolic activation, dimethylnitrosamine which had maximum toxicity and mutagenicity with a noninduced metabolic activating system, and 2-acetylaminofluorene which needed an induced metabolic activating system for mutagenicity.

A relationship between the mutagenic potency of carcinogenic compounds as measured in this system and their carcinogenic potency in rodents was constructed. This was in the form of a log-log plot, between carcinogenic potency expressed as frequency of tumor-bearing animals per μmole compound per kilogram body weight, and mutagenic potency expressed as mutagenic frequency per μmole compound-hr per ml. No extensive adjustments

were made to the carcinogenicity data for factors such as route of administration, duration of treatment or length of study. For example, the authors used the data of Druckrey et al. (16) to estimate the carcinogenic potency of methyl iodide, even though this study involved injection site sarcomas and less than lifetime exposure. The plotted points generally fell along a line with a slope of 1 and with deviations usually not more than $\pm$ 1 log unit. Thus, a linear relationship was found between these two measures which extended over 5-6 orders of magnitude in both carcinogenic and mutagenic potency. The authors concluded that with this type of relationship mutagenic potency could be used as a rough estimate of the carcinogenic potency of a compound in rodents.

Both types of TK mutations occurred. Both had an absence of thymidine kinase activity associated with the cells. Even slower growing mutants that would not grow in soft agar were also identified. These mutants were reported to have had gross chromosomal aberrations. These findings indicate that the fast growing mutants represent a point or gene mutation affecting the TK locus while the slow growing mutants may have heritable and viable chromosomal aberrations, which affect the TK locus and other genes.

Mutagenicity at the TK and HGPRT loci was examined for 13 compounds. For most of the compounds tested the TK locus was on the average nearly 10 times more mutable at 3 days post-treatment. Methyl iodide was mutagenic at the TK locus but

not mutagenic at the HGPRT locus. The period for expression was very important since the mutagenic frequency decreased after 48 or 72 hours (Table II-13). This decrease in mutagenic frequency was attributed to the formation of the two types of mutants, a fast-growing large colony mutant and a slow-growing small colony mutant. When the expression time was extended past 72 hours, the increasing number of fast-growing mutants may have diluted out the slower-growing mutants. For methyl iodide, the population of TK mutants had a bimodal distribution according to colony size, the number of small slow-growing colonies decreasing as expression time increased. The lack of effect at the HGPRT locus may have been due in part to the need for longer expression time to detect a mutation at that locus.

The mutagenic potency of methyl iodide in this system was 1.1×10^{-3} mutants/viable cell/μmole-hr/ml. On this scale, methyl iodide is almost as potent as methyl methanesulfonate and benzo[a]pyrene, and more potent than dimethylnitrosamine or 2-acetylaminofluorene. The mutagenic potency decreased by 1 to 2 orders of magnitude when the metabolic activating system was included in the assay. The dose-mutagenic response curve for methyl iodide was nonlinear and cytotoxicity was a problem, especially when the metabolic activating system was absent.

The characterization and validation of the mutagenicity assay in this study were carefully conducted. The positive

TABLE II-13

MUTAGENICITY OF METHYL IODIDE IN THE L5178Y/$TK^{+}/^{-}$SYSTEM

Locus Selection	Exposure (hours)	Metabolic Activating System	Expression (hours)	Concentration (µg/ml)	Survival (percent of control)	Mutagenic Frequency[a] ($x10^{6}$)
$TK^{+}/^{-}$; BUdR, 50 µg/ml	2.0	No	48	0	100	0
				6.8	63	54
				13.7	41	88
				23	7.7	229
				34	1.37	538
				46	0.12	697
			72	46	0.18	211
$TK^{+}/^{-}$; BUdR, 50 µg/ml	4.0	Yes	48	0	100	0
				30	75	-
				40	55	3
				50	41	43
				60	6.4	269
				70	0.39	-
$TK^{+}/^{-}$; BUdR, 50 µg/ml	4.0	Yes	144	0	100	0
				30	75	-
				40	55	-2
				50	41	49
				60	6.4	71
				70	0.39	110

[a]Number of induced mutants/10^{6} survivors after subrracting out background rates

SOURCE: Ref. 37

findings for methyl iodide were supported by the large number of assays conducted. The finding that methyl iodide may produce a mutagenic effect through a chromosomal mechanism is of interest since as a small molecule alkylating agent it is usually thought likely to act through a point mutational mechanism. This may mean either that the assay does not reflect the situation that would actually occur in vivo, or that methyl iodide can in fact induce chromosomal damage. The relationship shown in this study between mutagenic potency in this system and carcinogenic potency of a large number of compounds may be useful in estimating the oncogenic potency of compounds that have not been tested for carcinogenicity, but as the authors indicated, this is just a rough first estimate.

4. In Vivo and In Vitro Mutagenicity Screening of Methyl Bromide

McGregor (39) reported the results of five studies which examined the mutagenicity of methyl bromide. These mutagenicity studies made up the Tier II mutagenicity screen used by NIOSH to study 13 priority compounds including methyl bromide. The screen included an in vitro assay for unscheduled DNA synthesis using human embryonic cells, and four in vivo assays. Two of the in vivo assays (chromosomal aberrations in bone marrow cells and dominant lethal mutations) were conducted in rats, one assay (sperm abnormalities) was conducted in mice, and the last assay examined the frequency of recessive lethal mutation in Drosophila. All studies were done with methyl bromide derived from a single batch, but the purity was not specified.

Male and female rats (CD-Sprague Dawley) and mice (B6C3F1) were used in the mammalian studies. These animals were exposed to methyl bromide via inhalation. Exposure lasted 7 hours per day and 5 days per week if dosing was a multiple exposure. Ethyl methanesulfonate (EMS) was used as the positive control in most studies, and was administered by gavage.

In the unscheduled DNA synthesis assay, vinyl chloride gas was used as a positive control. This assay used human embryonic intestinal cells in passage 12-35. The cells were grown for 72 hours in normal medium that was then replaced with arginine-deficient medium for 24 hours. The cultures were divided into two groups, one of which had added to it a metabolic activating system (appropriate cofactors and the S-9 fraction from livers of rats pretreated with Aroclor 1254) while the other did not. The cells were exposed to airborne concentrations of 5, 10, 20, 30, 40, 50, 60, and 70% methyl bromide for 3 hours at 37°C. Negative controls were exposed to liquid DMSO. Unscheduled DNA synthesis was detected by the uptake of ^{3}H-thymidine into the nucleus of the cells while the cells were in a state of no DNA synthesis. Quantitation of uptake was effected by autoradiography after removal of unincorporated ^{3}H-thymidine.

Methyl bromide did not increase unscheduled DNA synthesis even at the 70% concentration. The values ranged from 3.8 to 12.1 grains/nucleus compared to the DMSO control, 5.2 grains/nucleus and showed no dose-response relationship. In contrast,

the positive control vinyl chloride was cytotoxic at airborne concentrations of 12.5%, 25%, and 50%, and increased unscheduled DNA synthesis at 12.5%.

The lack of cytotoxicity of methyl bromide is surprising because of its reactive nature. A good range of concentrations of methyl bromide was used, however, and the sensitivity of the assay appears to have been adequate to detect a effect if one had occurred.

In the cytogenetic analysis of bone marrow cells, groups of 10 male and 10 female CD-Sprague-Dawley rats were exposed to airborne concentrations of 20 and 70 ppm methyl bromide for 7 consecutive hours for a single exposure, or for 5 consecutive days for a multiple exposure. Bone marrow samples were taken at 6, 24, and 48 hours following the single exposure, and at 6 hours following the multiple exposure. A single or daily dose of 250 mg/kg ethyl methanesulfonate (EMS) by gavage was used as the positive control. The animals were treated with 3 mg/kg colchicine 4 hours after the last dose and 2 hours before they were sacrificed. (Colchicine administration should have been 2 hours prior to sacrifice for the animals sacrificed at 24 and 48 hours after dosing, but this was not indicated in the published report). Usually 50 cells, with a minimum of 41, were scored for each animal.

After multiple exposures to methyl bromide at 70 ppm, there was a significant increase in the number of aberrant cells from male rats, but not females. This significance was

lost when cells only containing chromosomal gaps were excluded from the analysis. Multiple exposures to 250 mg/kg/day EMS also significantly increased the number of aberrant cells from male rats, but not females. There was still a significant increase when cells with chromosomal gaps were excluded.

After single exposures, neither male nor female rats sampled at 6, 24, and 48 hours had an increased number of aberrant cells. Females sampled at 6 hours actually had significantly fewer aberrant cells than controls. This was probably a chance deviation in controls rather than a biological effect caused by methyl bromide. A single exposure to EMS produced significant increases in aberrant cells in males sampled at 6 and 24 hours, and in females sampled at 24 and 48 hours. Significance remained at the 24-hour sampling time for males and the 24- and 48-hour sampling time for females when chromosomal gaps were excluded.

In the dominant lethal assay, male CD-Sprague Dawley rats, in groups of 10, were also exposed to airborne concentrations of 20 or 70 ppm methyl bromide for 5 consecutive days. There was an unexposed control group and a positive control group given 5 daily doses of 100 mg/kg EMS by gavage. After treatment on day 5, two female rats were introduced to each cage holding an individual male rat. The females were removed after 5 days and were sacrificed 17 days after first introduction, an estimated 14 days after fertilization. New female rats were placed with the male rats every week for an additional 9 weeks.

When the females were sacrificed, their ovaries were examined for corpora lutea graviditatis and the uteri were opened to examine for live implantations, late deaths, and early deaths. The frequency of pregnancy was determined by (1) females with corpora lutea graviditatis and (2) females with implantations. With either method, methyl bromide was not found to cause a significant decrease in the frequency of pregnancy. EMS did produce a reduction in this frequency in weeks 2 and 3. The number of corpora lutea per pregnancy was also not affected by methyl bromide treatment, except in the 20 ppm group in week 7, but this was not considered biologically significant because there was no effect at 70 ppm. Significant ($p<0.05$) decreases were seen in the average number of corpora lutea per pregnancy in weeks 1, 3, and 9 in the EMS exposure group. Methyl bromide exposure did not affect the number of implantations per pregnancy, the frequency of live implantations, the combined frequency of live implantations and late deaths, nor the frequencies of one or more early deaths or two or more early deaths. The positive control EMS significantly decreased the number of implantations per pregnancy, the frequency of live implantations, and the frequency of live implantations and late deaths in weeks 1 through 4. The frequency of early deaths was significantly ($p<0.05$) higher than control at several time periods. Methyl bromide did not increase these frequencies except at 20 ppm in week 3. This again was not considered biologically significant, because of the lack of effect at 70 ppm.

For the sperm abnormality assay, groups of 10 male B6C3F1 mice were exposed to air containing 0 (controls), 20 or 70 ppm methyl bromide for 5 consecutive days. One group of 10 mice was given 5 daily doses of 200 mg/kg EMS. Five weeks after the last exposure, the animals were sacrificed, and the sperm in the cureda epididymides were isolated and prepared for examination. Sperm were then scored for abnormalities which were assigned categories: A, hook upturned or elongated; B, banana-shaped head; C, amorphous head; D, abnormal tail; E, miscellaneous. Methyl bromide did not increase the number of sperm abnormalities, while EMS significantly increased the number of sperm with amorphous heads.

The sex-linked recessive lethal assay was done in Drosophila melanogaster. Male Strain Oregon K (OrK) Drosophila from two different stock sources were exposed to airborne concentrations of 20 or 70 ppm methyl bromide for 5 hours. Positive control flies were exposed to EMS (0.4% in sucrose) for 5 hours. The following morning, each fly was mated with two virgin females of Muller-5 strain. The males were again mated with two new virgin females 3 days and 8 days after exposure. The F1 progeny from these matings were then mated, brother to sister, 1 to 4 days after emergence from pupae. The F2 generation was then examined for the absence of wild-type males. If no wild-type males were present, and there were eight or more Muller-5 males present in a vial, this was scored as a recessive lethal mutation. The F3 generation was also examined since this allowed for

the detection of mosaics or delayed mutations which might not have appeared in the F2 generation.

A dose-ranging study was first performed in order to determine the appropriate doses to use. Since no toxic effects were observed at the highest concentrations tested, 20 or 70 ppm, these were the concentrations used for the study. No toxic effects were observed in the main study, and the fertility was acceptable. At 20 ppm, the frequencies of lethal mutations in the F2 generation from one of the stocks were elevated. In broods 1, 2, and 3, they were 0.32, 0.81, and 0.27%, respectively. However, these were higher than those found in the F2 generation from this stock exposed to 70 ppm. Thus, the effect was not believed to be compound-related. EMS, in contrast, produced a frequency of 33.9% lethal mutations in the one brood of the F2 generation scored.

A common problem with the four in vivo studies in the testing screen is that the concentrations of methyl bromide used may not have been high enough to produce observable effects. There was no indication that preliminary studies were undertaken to determine the appropriate exposure concentration except in the recessive lethal mutation assay, in which no toxic effects were observed at 70 ppm. The approximate LC_{50} for mice is about 400 ppm for 7 hours, and in the study by Sikov et al. (16) female rats survived and reproduced without impairment despite exposure to 70 ppm methyl bromide for up to 40 days. These

facts suggest that higher levels could have been used. A positive finding was obtained in the chromosomal aberration assay, although the author discounted this finding because it became nonsignificant when chromatid and chromosomal gaps were removed as parameters in the analysis, and because it was observed only in males. In our judgment, however, this should be regarded as at least a marginally positive result in view of the fact that a similar pattern of effects was seen with the positive control compound. The results of the other three in vivo tests are not decisively negative because a maximum tolerated concentration was probably not achieved. They are best considered as giving no-observable-effect levels for these end points.

5. Study Utilizing the Pollen Tube Mitosis Assay

In an effort to extend the use of the pollen tube mitosis assay in Tradescantia from radiation to chemicals, Smith and Lotfy (40) selected methyl chloride as an example of an alkylating agent of low chemical reactivity, to be tested for its potential to induce chromosome damage. Ethylene oxide and ketene were also tested, and ultraviolet light (630 erg/mm^2/minute for 4 minutes) was used as a positive control. The pollen used was from clone B2-2 of Tradescantia paludosa with a haploid number of 6 chromosomes and a low frequency of fragments. Pollen was harvested, desiccated for 4 hours at 6°C, and treated for 5 minutes in a gas chamber (volume 650 ml) into which 6 or 7 ml methyl chloride were introduced from a pressure cylinder, giving nominal concentrations of 0.9% and 1.1%. Earlier studies had shown that treatment for 10 minutes with 5 ml or more of

methyl chloride was cytotoxic. The treated pollen was cultured on a medium containing 1% agar, 12% lactose, and 0.01% colchicine, which was in a thin layer on a cover glass (amounts of pollen and medium unspecified). The cover glass was inverted on a slide over a small piece of moist paper to form a humidified cell. These cells were incubated at 25°C until metaphases were present, about 24 hours for treated pollen and 2 to 3 hours less for the controls. Permanent slides stained with acetocarmine were prepared. The report did not make clear how many treatment replicates were run or how many slides were prepared from each batch of treated pollen. The slides were scored for chromatid breaks, abnormal chromosome condensation, and "eroded" chromosomes (those with an "eaten away" appearance, but still continuous). Exactly how the chromosomes in particular pollen tubes were selected for scoring was not stated.

Control cultures yielded 6 breaks among 6,590 chromosomes (0.09%), with no erosions or contractions. Both concentrations of methyl chloride produced a significant increase in aberrations: 240 breaks in 5,932 chromosomes (4.04%) at 0.9%, and 93 breaks in 3,008 chromosomes (3.09%) at 1.1%. A similar pattern of aberrations was produced by ultraviolet light: 126 breaks in 2,533 chromosomes (4.97%). The other two chemicals tested yielded different patterns of aberrations: fewer breaks and significant increases in erosions and contractions. Although the effects produced by methyl chloride were clearly positive, the dose-response relationship appeared reversed between the

two tested concentrations. However, the nominal concentrations were similar and actual concentrations were not measured, so this does not appear to be a major flaw in the experiment.

The demonstration that chloromethane breaks the resting chromosomes of Tradescentia pollen in a radiomimetic fashion supports the other evidence that the substance is genotoxic. The results of the study appear valid, but several methodological details were not reported, and the actual doses of methyl chloride to which pollen was exposed could not be readily determined. This plant assay has not been retained in batteries of short-term tests currently employed to screen for genotoxicity, so that its usefulness in assessment of mutagenic and carcinogenic potential is limited.

6. Study of Methyl Bromide for DNA Alkylation and Mutagenicity in E. Coli

Djalali-Behzad et al. (13) attempted to correlate the degree of in vitro and in vivo alkylation of hemoglobin by methyl bromide to that of liver and spleen cell DNA, in order to estimate a factor that could be used in calculating the genetic risks posed by methyl bromide. In addition, the authors examined the activity of methyl bromide in a forward mutation assay in E. coli in order to establish relationships between dose, degree of DNA alkylation, and mutagenic response.

The mutagenic effect of methyl bromide on E. coli Sd-4, a streptomycin-dependent strain of E. coli B, was studied by exposing bacteria to methyl bromide at 37°C for one hour while they were suspended in liquid medium. Dose was calculated

as the concentration of methyl bromide in the medium times the length of exposure (mM-h). The exposed bacteria were either grown on plates containing streptomycin and the colonies counted 24 hours later to determine survival, or they were grown on plates without streptomycin and counted 72 hours later to determine mutagenicity.

Methyl bromide was mutagenic to this strain of E. coli. Its mutagenic efficiency (maximal mutation rate attainable) was calculated from the dose-response curve and found to be about 1 mutation per 10^8 surviving bacteria per mM-h. Compared to those for other mono-functional alkylating agents, this mutagenic efficiency was found to be 2-5 times less than expected on the basis of rate constants for reaction with a nucleophilic center of low nucleophilic strength. This result suggested that the concentration of methyl bromide in the environment of the bacterial DNA may have been lower than that in the liquid medium.

In order to investigate alkylation of protein and DNA by methyl bromide, the formation of specific alkylation products in protein and DNA was measured. The products included S-methylcysteine and N^{π}- and N^{τ}-methylhistidine for proteins and N-7-methylguanine for DNA. In vitro alkylation of protein was examined in the hemoglobin of red blood cells and the protein of spleen cells from CBA mice, and in vitro alkylation of DNA was examined in mouse spleen cells and also in isolated DNA. The isolated DNA was suspended in phosphate-saline buffer during

exposure. Exposure was to labeled ^{14}C-methyl bromide (specific activity 4.9 to 5.0 mCi/mmole, radioactive purity >98%) at initial concentrations of 57 μCi/liter (11.4 μM), 83 μCi/liter (16.6 μM) and 19 μCi/liter (3.8 μM) for the DNA, spleen cells, and red blood cells, respectively. Measurements of radioactivity in solution were made before and after 60 minutes incubation for DNA and red blood cells and after 34 minutes for spleen cells at 37°C. There was some loss of methyl bromide during the incubation which was attributed to evaporation and not to a chemical reaction in the buffer.

The rate constants for alkylation of cysteine-S and histidine N^{π} and N^{τ} in hemoglobin were $6x10^{-4}$, $2x10^{-5}$, and $3x10^{-6}$ liters/g Hb/hour, respectively. The rate constant for alkylation of histidine-N^{π} in hemoglobin was similar to that of histidine in protein of spleen cells, $3x10^{-5}$ liters/g protein/hour. The rate constant for alkylation of guanine-N-7 in isolated DNA was an order of magnitude higher than that in DNA of spleen cells, $3x10^{-4}$ and $4x10^{-5}$ liters/g DNA/hour, respectively. The authors found this difference interesting, since a difference in rate constants for alkylation of DNA was not seen for alkylation of hemoglobin in red blood cells and protein in spleen cells. As with the bacteria, the concentration of methyl bromide in the buffer solution must have been greater than that found in the compartments of DNA.

The in vivo study on the degree of alkylation of hemoglobin, liver cell protein, and liver and spleen cell DNA by methyl

bromide was conducted on mice following exposure via inhalation or intraperitoneal injection. Methylation of cysteine and the N-7 position of guanine were used as measures of alkylation of protein and DNA, respectively. Two groups of 9 male CBA mice 6 to 8 weeks of age were exposed in an 11 liter all-glass system. They were exposed to an atmosphere initially containing 80 μCi (16 μmoles) and 37 μCi (7.4 μmoles), respectively, which was equivalent to about 140 and 65 μg/liter, respectively. The half-life of methyl bromide in the chamber was determined to be 30 minutes. Exposure ended after 4 hours and the animals were killed and tissues taken for analysis. Seven mice of a third group were injected with 4.4 μmoles of methyl bromide (21.6 μCi per kilogram body weight). They were sacrificed 5 hours after injection and tissues were removed for analysis.

The administered doses of methyl bromide given by inhalation were reported to be 340 μCi/kg (6.5 mg/kg) and 174 μCi/kg (3.3 mg/kg) although it was not indicated how these doses were calculated. At the higher dose, alkylation of hemoglobin cysteine residues by methyl bromide was measured to be 2.2×10^{-2} μCi/g protein. In the liver protein, alkylation was 1×10^{-3} μCi/g protein, which was about 20 times lower than what was expected since the in vitro study suggested that protein alkylation should be similar between hemoglobin and other tissues. Alkylation of liver and spleen DNA were expected to be one-half that found in hemoglobin based on the in vitro study. They were, however, 5×10^{-5} and 5×10^{-4} μCi/g DNA, respectively, which

were 5,000 times and 500 times, respectively, lower than expected. Only alkylation of hemoglobin was determined in the other two in vivo studies. The ratios between administered dose and alkylation of hemoglobin were similar in the two inhalation studies and the injection study, suggesting that alkylation of hemoglobin may be a reasonable measure of dose.

The authors found it interesting that the rate constant for alkylation of DNA in suspended spleen cells was only 12% of that found in isolated DNA, while alkylation of hemoglobin and spleen cell protein were the same. They failed to discuss the fact that both measures of protein alkylation were in cells while this was not true for DNA. These in vitro data are useful in elucidating the results of the in vivo study. Although the authors discussed how alkylation of liver protein and liver and DNA in vivo were lower than expected from hemoglobin alkylation, they did not compare alkylation of liver protein to alkylation of DNA. In this case, alkylation of liver DNA was 20-fold less than liver protein alkylation, which compares nicely to the 15-fold difference between in vitro alkylation of hemoglobin and alkylation of spleen cell DNA. Therefore, such studies in in vitro and in vivo systems may be useful in the estimation of mutagenic risk, if a parameter such as hemoglobin alkylation is used as a measure of effective dose.

The main purpose of this study was to determine whether a factor could be estimated which would be useful in determining genetic risk based on exposure information. The empirical

basis of this hypothesis was that in forward mutation systems of E. coli and barley, the degree of alkylation of nucleophilic centers is proportional to the mutation frequency, and that a degree of alkylation of 10^{-7} corresponds to the effect of 1 rad of gamma radiation. This factor also was reported to be valid for in vivo rodent mutagenicity systems. The authors used this result to calculate the mutagenic risk posed by a chemical in radiation equivalents. The results of the present experiment showed that for methyl bromide, a correction factor, less than 1, has to be applied to take into account the difference between the dose in red blood cells and the dose in the components of DNA.

There are several technical problems in the reporting of this investigation which make it difficult to use its results in comparison to other studies. The problems include lack of a specific detailed methodology, as in the mutagenicity study where the exposure concentrations were not given. It was also indicated that methyl bromide was lost during the incubation despite the fact that the incubation tubes were reported to be tightly capped. Because of this loss, effective exposures had to be calculated but were not reported. The method used to calculate mutagenic efficiency was also not specified, and this parameter cannot be calculated from the presented data, yet it was the basis of an important comparison. Additionally, it was also not indicated how the rate constants for alkylation of cysteine, histidine and guanine were calculated for the

in vitro study, nor was it indicated how the administered dose of methyl bromide was calculated for the two inhalation studies.

Very important omissions from the methodology were detection limits for adduct formation. The data presented suggest that in vivo adduct formation would be detectable in protein; however, measurement of in vivo DNA adduct formation at the levels reported (less than 1 pCi per mouse) is improbable. Thus, the data from this study are not likely to be useful to estimate genetic risk without more details of methodology. However, this study does provide convincing evidence that methyl bromide is mutagenic in E. coli under the conditions of the test.

E. Summary and Evaluation of Evidence for Potential Carcinogenicity

In this section of the report, we summarize the evidence provided by the studies reviewed in foregoing sections for the potential carcinogenicity of monohalomethanes.

1. Methyl Chloride

Methyl chloride is the only one of the three compounds under review that has been subjected to a full-scale carcinogenesis bioassay according to modern protocols. This study (14) gave reasonably convincing negative results for carcinogenicity of inhaled methyl chloride in Fischer rats and female B6C3F1 mice. However, it gave sufficient evidence that inhaled methyl chloride is carcinogenic in male B6C3F1 mice, causing a dose-related increase in kidney tumors and probably liver tumors, as well as a spectrum of related pathological lesions in the

kidney and liver. The kidney tumors included both benign and malignant types and were observed in male mice exposed both to 1,500 ppm and to 225 ppm methyl chloride. There was no clear evidence that the dose-response curve was markedly non-linear nor of association of kidney tumors with specific types of tissue damage.

One thorough study of the teratogenicity of inhaled methyl chloride (21, 22) gave negative results for Fischer rats, although the sensitivity of this study was limited. In the same study, inhaled methyl chloride was teratogenic in B6C3F1 mice, causing a characteristic type of heart defect in offspring of animals exposed to concentrations of 750 ppm or 500 ppm. This finding provides little additional information about the potential carcinogenicity of methyl chloride, except to show that it can be transported to sensitive target organs and affect developing cells.

Two independent studies (25, 26, 29) have shown that gaseous methyl chloride induces base-substitution mutations in S. typhimurium (strains TA 1535 and TA 100) in the Ames assay. In both studies, the effect was noted in the absence of metabolic activation and was slightly (although barely significantly) increased in the presence of metabolic activation. This suggests that methyl chloride is a direct-acting mutagen in this system, and that its metabolites either are mutagenic also or are formed too slowly to reduce the measured effect. These results indicate that methyl chloride has the potential to act as a carcinogenic

initiator if it or its metabolites can reach the target cells. The only other mutagenicity study on methyl chloride is an early study (40) which showed that it causes chromosome damage in Tradescantia paludosa. Although this study provides sufficient evidence of genotoxicity in this system, the assay has not been standardized or validated, and its relevance to the potential carcinogenicity of methyl chloride is unclear.

In summary, methyl chloride has been shown to be carcinogenic in male mice exposed by inhalation to concentrations of 225 or 1,000 ppm. It is a direct-acting alkylating agent, a direct-acting mutagen and possibly an indirect-acting mutagen. As such, it is likely to be a carcinogenic initiator if it can reach target tissues. There is no evidence that it induces cancer in mice by an indirect mechanism or that its dose-response relationship is markedly nonlinear. These findings are limited by the meager evidence available on mutagenicity or mechanisms of action. The only reason for questioning the general applicability of the finding of carcinogenicity is that it was limited to male mice. Reasons for the lower susceptibility of female mice and male and female rats are not known.

2. Methyl Iodide

Only two direct studies of the potential carcinogenicity of methyl iodide have been reported. One study (15, 16) showed that subcutaneous injections of methyl iodide at doses of 50 mg/kg (single dose) or 500-900 mg/kg (repeated doses) into rats led to a high incidence of sarcomas at the injection site (and

perhaps at distant sites). Another study (18) suggested that intraperitoneal injections of methyl iodide at total doses up to 44 mg/kg into strain A mice led to an increase in pulmonary tumors, but the experiment was insensitive and the result was not statistically significant. However, other alkyl iodides gave positive results in the same experiment. Although neither type of assay can provide sufficient evidence of carcinogenicity, there is a fairly good correlation between the induction of injection site sarcomas and positive results in full-scale carcinogenesis bioassays (41). Thus, the study reported in Druckrey et al. (16) provides at least limited evidence of carcinogenicity of methyl iodide.

Methyl iodide is a direct-acting alkylating agent and has given clear positive results in two of three reported tests for mutagenicity. In one study (25, 26) it induced base-substitution mutations in the Ames assay without metabolic activation. In another study (37) it was found to be a potent mutagen in the L51787 mouse lymphoma assay. However, it gave negative results in the Neurospora back mutation assay (33). Despite this negative result, methyl iodide appears to be a direct-acting mutagen in at least two systems and thus has the potential to act as a carcinogenic initiator.

In summary, there is limited evidence for the carcinogenicity of methyl iodide in vivo and strong evidence that it acts as a direct-acting mutagen. Despite the absence of an adequate full-scale bioassay, there is a strong probability that methyl

iodide would be carcinogenic in animals exposed via inhalation, provided that it is able to reach target tissues prior to being metabolized and detoxified.

3. Methyl Bromide

Methyl bromide has not been tested for carcinogenicity in mammals, and data on its mutagenicity and other related effects are conflicting. One study (25, 26) showed that it was a potent mutagen in the Ames test without metabolic activation, and another study (28), although questionable because of poor reporting, gave similar results. A third study (13) showed that it was mutagenic in E. coli and that it was capable of alkylating DNA both in vitro and in vivo after exposure of mice by inhalation. However, another series of studies (39) gave negative results in a test for unscheduled DNA synthesis, marginally positive results for induction of chromosomal aberrations in rats, and negative results in three other in vivo assays (dominant lethal assay in rats, sperm abnormality in mice, and sex-linked recessive lethal assay in Drosophila). In addition, negative results were reported in teratogenicity studies in rabbits and rats (24). All the in vivo studies were limited in sensitivity by the high toxicity of methyl bromide, and most of them were probably further limited by failure to achieve a maximally tolerated dose. Thus, they provide only limited evidence to offset the other positive results. The potency of methyl bromide as a direct-acting alkylating agent (17) and a direct-acting mutagen in Salmonella

(25, 26) suggests that it could act as a potent carcinogenic initiator if it were to reach the target tissues. However, its reactivity suggests that it is more likely than methyl chloride to be rapidly detoxified after absorption into the body (15, 17). This may explain the negative results of teratogenicity and mutagenicity tests in vivo at modest exposure levels (24, 39). However, one study showed that methyl bromide can alkylate DNA in vivo, although to a lesser degree than predicted in view of its alkylation efficiency in vitro (13).

The weight of the evidence cited above suggests that methyl bromide is very likely to be carcinogenic in animals exposed via inhalation. In view of its reactivity, it may be more likely than methyl chloride to act in the lung or other sites of first contact.

F. Dose-Response Information

Only meager dose-response information is available that can serve as the basis for estimation of the carcinogenic potency of monohalomethanes, and hence, as the basis for carcinogenic risk assessments.

1. Methyl Chloride

The data summarized in Tables II-3 and II-4 provide direct measures of the dose-response relationship for carcinogenic effects of inhaled methyl chloride in male B6C3F1 mice. As such, they can be used directly in risk assessment (see Section V below). However, as explained in Section II.A.15 above, utilization of the data is complicated by the temporal distribution

of deaths in the animals exposed to 1,000 ppm, and the other exposure concentrations were set too low to provide much useful statistical information. No other useful dose-response data for methyl chloride are available.

2. Methyl Iodide

The only dose-response data for carcinogenic effects of methyl iodide are the results of the study by Druckrey et al. (16). However, it is very difficult to use these data for risk assessment, since most or all of the induced cancers were of sarcomas at the site of injection, and the local tissue doses were indeterminate and presumably very high. Although Clive et al. (37) used these data to estimate the carcinogenic potency of methyl iodide as lying between 10^{-3} and 10^{-4} tumor-bearing animals/μmole/kg body weight, such an estimate is not comparable with estimates of potency derived from experiments in which animals are exposed by ingestion or inhalation. Hence, it is of little or no use in risk assessment.

The only other dose-response data on methyl iodide derive from genotoxicity studies. In the mouse lymphoma assay, its mutagenic potency was estimated as 1.1×10^{-3} $TK^{+/-}$ mutants/cell/μmole-hour/ml in the absence of metabolic activation (37). This placed methyl iodide intermediate in potency between benzo[-a]pyrene and dimethylnitrosamine. However, in this assay, its mutagenic potency was reduced by factors of 8-40 by metabolic activation to levels similar to those of ethylene dibromide and 2-acetylaminofluorene (37). If mutagenic potency can be

used as a rough predictor of likely carcinogenic potency (as suggested in reference 37), the lower figure would be more appropriate to use if methyl iodide is assumed likely to act at a distant site in the body, but the higher would be appropriate if it is assumed likely to act at the site of first contact.

The only other numerical dose-response data are those reported in references 25 and 26, which indicated that methyl iodide was about 11 times more potent (on a volume/volume basis) than methyl chloride when tested under similar conditions in the Ames assay. These tests were conducted in the absence of metabolic activation, and the difference in biological activity between the chemicals would be expected to be reduced if methyl iodide were subjected to metabolism (e.g., after passage through the liver to distant organs in the body).

3. Methyl Bromide

The only useful data on the biological activity of methyl bromide are those reported in references 25 and 26, which indicated that methyl bromide was about 39 times more potent (on a volume/volume basis) than methyl chloride when both were tested under similar conditions in the Ames assay in the absence of metabolic activation. The data reported in reference 30, although questionable because of poor reporting, suggest that metabolic activation would not decrease and might increase the activity of methyl bromide.

The study reported in reference 13 yielded a number of relationships between degree of alkylation of DNA and dose

in vivo or in vitro. However, it is not possible to use these data in risk assessment without an empirical relationship between degree of alkylation of DNA and carcinogenic response. The only data from reference 33 that may be useful in risk assessment are the results for mutagenic effectiveness in E. coli, which indicated that methyl bromide (in the absence of metabolic activation) is 6.5-13 times less potent than ethylmethanesulfonate (EMS) and 11-22 times less potent than methylmethanesulfonate (MMS) in this system.

4. Summary

Quantitative data on the dose-response relationship for induction of cancer in animals are available only for methyl chloride. For methyl bromide and methyl iodide, the only available data are measurements of their mutagenic potency relative to those of methyl chloride and other mutagens in various assays for mutagenicity. The usefulness of these data is limited by incomplete knowledge of the quantitative relationships between mutagenic potency and carcinogenic potency.

III. METABOLISM AND PHARMACOKINETICS

A. Introduction

Information on the pharmacokinetics and metabolism of compounds can provide two important types of information for quantitative risk assessment. Comparative pharmacokinetic data are useful in scaling exposure levels from animal data to humans. Data on pharmacokinetics and metabolism can also be used to indicate whether a compound will be handled by animals in a similar manner at low and high exposure levels. Data on the metabolism of a compound and its binding to DNA and other macromolecules may also indicate what the active agent is and will permit the estimation of scaling factors if there are differences in binding or metabolism between the animal model and humans. In particular, for the monohalomethanes, data on both pharmacokinetics and metabolism may be useful in determining relative biological activity. This section of the report reviews the available studies of metabolism and pharmacokinetics of monohalomethanes that are useful in risk assessment.

B. Methyl Chloride

The absorption of methyl chloride via inhalation has been studied by Andersen et al. (42). These investigators used a recirculating closed system to determine the kinetics of uptake by rats. Measurements were made of the loss of methyl chloride from the closed system over time at several different

initial airborne concentrations. The rate of loss of methyl chloride from the air was determined for each concentration and plotted. Andersen et al. concluded that uptake of methyl chloride could be described by a mixed kinetic process. There appeared to be both saturable and nonsaturable components to the uptake. This was manifested as a decrease in the apparent first order rate constant of uptake with increasing airborne concentration, until a point was reached where the rate constant no longer changed and uptake became a first order rate process. The rate of uptake was described mathematically using Michaelis-Menten kinetics for the saturable portion of the uptake and first-order kinetics for the nonsaturable portion of the curve. The equation used was:

$$\text{Rate} = \frac{V_{max}\ [c]}{K_m + [c]} + k^1[c],$$

where V_{max} = 7.7 mg/kg/hr and was the maximum rate of uptake from the 31-liter chamber, [c] was the concentration in ppm, K_m = 630 ppm and was the Michaelis-Menten constant, and k^1 = 0.027/kg/hr and was the first-order uptake rate constant.

Several other halogenated hydrocarbons studied in this system also showed mixed uptake kinetics. The investigators suggested that the uptake of these compounds could be described by a four-compartment pharmacokinetic model, in which compartment one was the gas, compartment two was the blood and richly perfused tissues (e.g., liver and kidney), compartment three was the poorly perfused tissues (e.g., fat), and compartment four

was made up of nonvolatile metabolites. The saturable component of the uptake curve could result from saturation of an enzymatic process such as metabolism, or from slow transfer into poorly perfused tissues. The first-order component of the uptake could result from rapid transfer of the compound from the blood and richly perfused tissues to poorly perfused tissues, or it could result from nonenzymatic metabolism.

This study suggested that a saturable process may occur in the biological handling of methyl chloride by rats. If so, there would not be a direct proportional corrrelation between exposure concentrations and tissue levels. However, there are several problems with this study that prevent acceptance of this conclusion without reservation. First, the measurements reported were of the rate of decrease of the chamber concentration of methyl chloride, and thus provided only indirect measures of uptake. Second, no actual data were presented for methyl chloride; even the test concentrations were not reported. Third, the data appear to have been obtained from only one run at each of five test concentrations. The only statistical analysis reported was a correlation coefficient ($r^2 = 0.90$) for a linear regression in an Eadie-Hofster plot; no confidence limits were given for the parameters of the saturable portion of the loss curve.

Landry et al. (43) also examined the pharmacokinetics of methyl chloride in rats as well as in dogs. These investigators used a nonrecirculating system in which the airborne

concentration of methyl chloride was kept constant. The levels of methyl chloride in blood were measured by gas chromatography over the 6-hour exposure period for rats and 3-hour exposure period for dogs. Postexposure blood levels were also determined. At both airborne concentrations used, 50 and 1,000 ppm, blood levels quickly attained steady-state levels. The postexposure blood levels indicated that there was a rapid biphasic decline. Steady-state blood levels were similar in the rat and dog while the elimination was more rapid in the rat than the dog. A linear two-compartment model was developed which accurately described the pharmacokinetics of methyl chloride in these animals. This model only differed from the four-compartment model of Andersen et al. (42) in that the gas was not considered a compartment since this exposure system was not closed, and the metabolite pool was not considered a compartment. The pharmacokinetic parameters were determined and are tabulated in Table III-1.

The investigators noted that in the dogs higher respiratory rates lead to higher end exposure blood levels of methyl chloride. Ratios between steady-state blood concentrations and exposure concentrations were similar at both exposure concentrations and in both species. This indicates that at concentrations up to 1,000 ppm there was no apparent saturation of uptake or intercompartment transfer.

Von Oettingen et al. (44) exposed dogs to an airborne concentration of 15,000 ppm methyl chloride. The exposure

TABLE III-1

PHARMACOKINETIC PARAMETERS FOR METHYL CHLORIDE IN RATS AND DOGS

Animal	Exposure concentration (ppm)	K_o[a] ng/g/min	K_{12}[a] (min^{-1})	K_{21}[a] (min^{-1})	K_{1E}[a] (min^{-1})	End-exposure blood concentration (ng/g)	AUC[b] (μg-min/g)	α-$t_{1/2}$ (min)	β-$t_{1/2}$ (min)
Rats (n=3)	50	28.5	7.6×10^{-3}	5.0×10^{-2}	15.0×10^{-2}	194	69.7	4.4	14.8
Rats (n=3)	1000	540.0	7.5×10^{-3}	4.9×10^{-2}	14.0×10^{-2}	3930	1415.0	4.7	15.2
Dog 7609	50	9.9	9.4×10^{-3}	2.3×10^{-2}	7.3×10^{-2}	135	24.5	8.1	35.4
Dog 7382	50	15.9	17.1×10^{-3}	1.7×10^{-2}	8.8×10^{-2}	177	32.4	6.4	51.0
Dog 7935	50					171[c]			
Dog 7935	1000	190.0	5.6×10^{-3}	2.0×10^{-2}	5.9×10^{-2}	3220	583.0	10.4	40.0
Dog 8061	1000	282.0	9.0×10^{-3}	3.1×10^{-2}	6.9×10^{-2}	4080	735.0	8.3	27.0
Dog 7382	1000					3760[c]			

[a] K_o rate constant for uptake; K_{12} and K_{21}, rate constants for transfer between compartments 1 and 2; K_{1E}, rate constant for excretion

[b] Integrated area under blood concentration vs. time curve

[c] Means of blood concentrations at end of 3-hr exposure

SOURCE: Ref. 43, Table 1

system was nonrecirculating like that of Landry et al. (43), but the dogs were anesthesized during the exposure. Even though there were differences in protocol, the ratio of steady-state blood concentration to exposure concentration was very similar to those found by Landry et al. (43) for dogs (Table III-2). This suggests that the kinetics of methyl chloride in dogs are nonsaturable up to at least 15,000 ppm.

The last column of Table III-2 shows the ratio between the uptake rate constants and the end-exposure blood concentration. These ratios were similar at exposure concentrations of 50 and 1,000 ppm in each species, again indicating no saturation of uptake kinetics. In contrast, the data reported by Andersen et al. (42) predict only a 10-fold increase in uptake rate between concentrations of 50 and 1,000 ppm. Because Landry et al. used direct measurements of uptake and reported their results in detail, their results appear much more convincing.

In addition to the pharmacokinetic study, Landry et al. (43) measured tissue levels of nonvolatile ^{14}C-radioactivity and nonextractable ^{14}C-radioactivity following 6 hours exposure of rats to air concentrations of 50, 225, 600, and 1,000 ppm ^{14}C-methyl chloride. Liver, kidney, and testis levels of nonvolatile radioactivity increased proportionally to air concentration, again suggesting nonsaturable kinetics. However, tissue levels of nonextractable radioactivity did not increase proportionally and, in fact, appeared to plateau in the kidney between exposure concentrations of 225 and 1,000 ppm (Figure III-1).

TABLE III-2

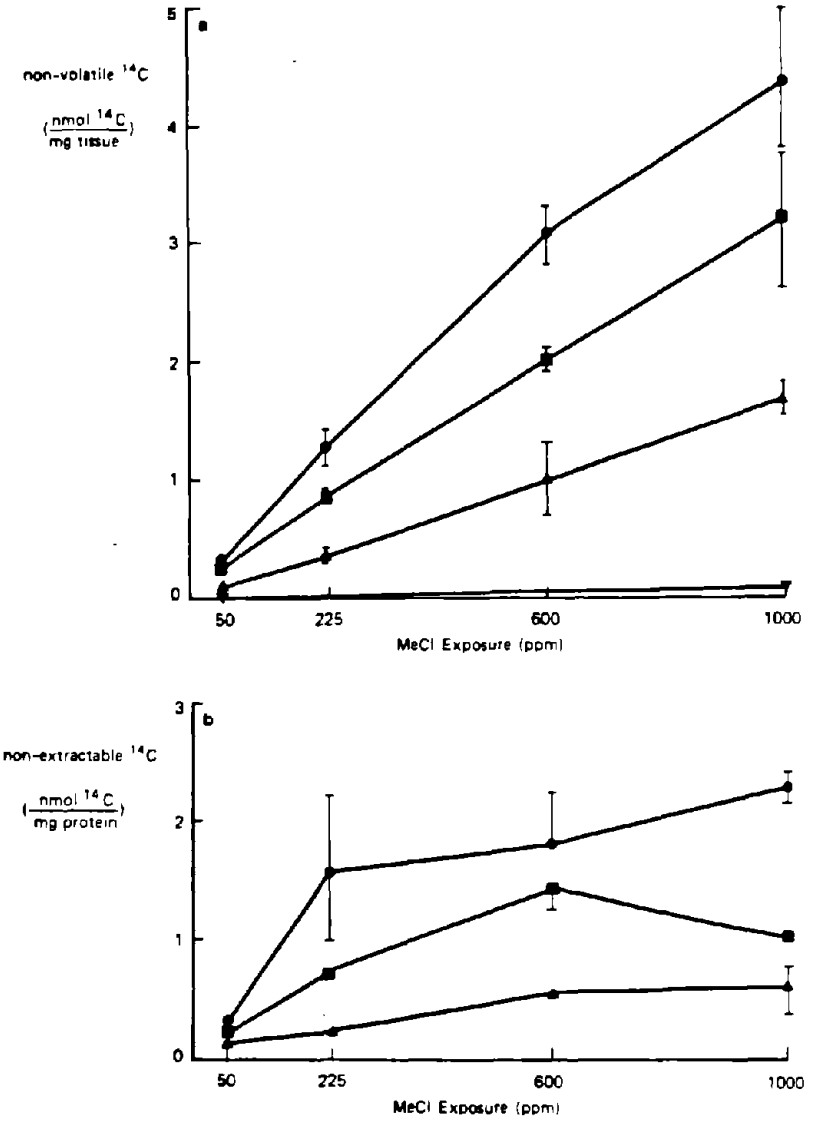
COMPARISON OF PHARMACOKINETIC PARAMETERS FOR METHYYL CHLORIDE

Species	1 Exposure Concentration (ppm)	2 End-exposure Blood concentration (ng/g)	3 Uptake Rate constant (ng/g/min)	Ratio 2/1	Ratio 3/1
Rat	50	194[a]	28.5[a]	3.9	0.57
	1000	3930[a]	540.0[a]	3.9	0.54
Dog	50	161[a]	12.9[a]	3.2	0.26
	1000	3687[a]	236.0[a]	3.7	0.24
	15000	60600[b]		4.0	

[a]From Ref. 42
[b]From Ref. 43

FIGURE III-1

NONVOLATILE AND NONEXTRACTABLE RADIOACTIVITY IN RAT TISSUES FOLLOWING EXPOSURE TO [^{14}C] METHYL CHLORIDE



Total (nonvolatile) ^{14}C (a) and nonextractable ^{14}C (b) in liver (●), kidney (■), and testis (▲) of rats immediately following 6 hour [^{14}C]MeCl exposures. (Values are $\bar{\chi} \pm$ SD, n = 6). End-exposure blood MeCl (parent compound) concentration in 50 and 1,000 pm exposed rats is also shown (▼).

SOURCE: Ref. 43, Figure 4

This does suggest a saturation in the pathways which lead to the binding or incorporation of ^{14}C-methyl chloride into the tissues, although the effect was clearly significant only in the kidneys.

A few studies on uptake and excretion of methyl chloride have been done using human volunteers. Hake et al. (45) exposed adult males and females to different air concentrations for varying lengths of time. They noted that the group could be divided into two subgroups, one subgroup having blood and breath levels of methyl chloride 2 to 6 times higher than the other subgroup. This study was reported in abstract form and so only limited information was presented. However, it does suggest that a large variation in blood concentrations might be expected in a population exposed to the same air concentration.

Morgan et al. (46) reported a more extensive study on the excretion of methyl chloride, as well as many other chlorinated aliphatic compounds, in the exhaled breath following inhalation. The investigators used ^{38}Cl-labeled methyl chloride for this study and each subject inhaled approximately 5 mg of material (500 ppm) in a single breath. The subjects held their breath for 20 seconds and then exhaled through charcoal traps to remove unabsorbed methyl chloride. Twenty-two percent of the methyl chloride was unabsorbed as measured by this procedure. This large amount of unabsorbed compound was expected because of the low blood-air partition coefficient for methyl chloride.

The rate of excretion of methyl chloride was determined at different time periods after administration. This rate decreased quite rapidly over a 60-minute time period. The total excretion of methyl chloride after 1 hour was reported as 29% of the dose. (It is not clear whether this is based on retained or delivered dose.)

Using a log versus log scale, the investigators plotted the amount of compound retained against time. This yielded a straight line. Plotting the slope of this line for the other chlorinated compounds studied versus their respective partition coefficients in a log-log plot gave a very good correlation. The position of methyl chloride, however, was clearly anomalous in this correlation. The investigators suggested that this anomaly could result from breakdown of methyl chloride in the blood, which would decrease its concentration and thus its excretion rate. (Although they proposed that this breakdown would occur in the blood, breakdown anywhere in the body would give the same result.) Since the calculation of retention was based on measurements of the amount of the compound exhaled, this effect would make it appear that the retention of methyl chloride is longer than would be predicted by the partition coefficient. When the log of the excretion rate, expressed as a percentage of "contemporary body content" (an undefined term), was plotted against the log of time after administration, a linear relationship was obtained. This was done for the family of chlorinated methanes. The slopes of the lines for

the compounds other than methyl chloride were similar while the slope of the line for methyl chloride was steeper. This again suggested that methyl chloride was being broken down in the body.

The excretion pattern for methyl chloride found in this study may well differ from that which occurs under prolonged exposure. This study, however, does show that inhaled methyl chloride is partially exhaled and that there is probably some breakdown of methyl chloride in the body. The authors suggested that this breakdown is due to a chemical reaction with the blood, but gave no specific reason for this suggestion.

Redford-Ellis and Gowenlock (47) studied the reaction of methyl chloride with blood and blood components. Methyl chloride failed to react with serum components saline, glucose, mixed plasma lipids, many different amino acids, or glutathione. Serum and plasma, however, did take up methyl chloride, although the methodology used to determine uptake was not clearly described. When ^{14}C-labeled methyl chloride was used, plasma firmly bound radioactivity. The amount of activity bound accounted for only 1 to 2% of the methyl chloride taken up by plasma as determined by the previous method. The investigators suggested that this difference might be the result of the formation of volatile compounds which would go undetected by the previous method. Analysis of the protein binding indicated that 98.4% of the activity was associated with S-methylcysteine and the rest was associated with 1- and 3-N-methylhistidine.

When erythrocytes were incubated with ^{14}C-methyl chloride, radioactivity was found to bind to a soluble compound and this complex would not diffuse out of the cell. The bound compound was identified as S-methylglutathione. When the erythrocytes were heated at 120° (temperature scale unstated but presumably F) for 30 minutes before incubation with methyl chloride, binding was reduced by 90%. This indicates that enzymatic binding accounted for the majority of binding that occurred, which is supported by the fact that glutathione did not chemically react with methyl chloride in plasma. It is not clear from this report whether the investigators considered protein binding in plasma to be caused by a chemical or enzymatic reaction.

The same investigators (48) also examined the reaction of ^{14}C-methyl chloride with homogenates of liver, brain, and kidney tissue from rats and guinea pigs. Radioactivity was bound in liver tissue homogenate. Binding was found to be to soluble components of the homogenates and these compounds were identified as S-methylglutathione and S-methylcysteine. The binding was reduced by 98-99% when the tissue was first heated at 120°[F] for 30 minutes. In kidney and brain homogenates, binding to glutathione and cysteine also occurred. In addition, binding to cysteine residues of protein was observed in these tissues. In these two tissues heat treatment also substantially decreased binding of radioactivity. This suggests that most or all binding is mediated through an enzymatic process.

Tissue nonprotein sulfhydryls (NPSH), which are mainly made up of glutathione, were depleted when rats were exposed to methyl chloride via inhalation (49). When rats were exposed to airborne concentrations of 500 or 1,000 ppm for 6 hours, significant decreases in the levels of NPSH occurred in liver, kidney, and lung. NPSH levels in liver and kidney returned to normal 8 hours following exposure, while it took longer for the levels to return to normal in lung tissue. Exposure to 100 ppm methyl chloride did not significantly depress tissue NPSH levels. The NPSH level in blood was not significantly depressed by methyl chloride exposure. This was surprising since in an earlier study (47) an interaction between methyl chloride and erythrocyte glutathione was observed. The large pool of glutathione in erythrocytes may not have been significantly depleted by the exposures.

Landry et al. (43) have also shown that inhalation of methyl chloride depletes tissue NPSH in a dose-related fashion. In this study, rats exposed for 6 hours to an airborne concentration of 225 ppm or more had significantly reduced NPSH levels in liver, kidney, testis, and epididymis. These investigators examined the urinary excretion of methyl chloride metabolites. The excretion of radioactivity following exposure to ^{14}C-labeled methyl chloride increased with increasing exposure concentration. There was no indication of a saturation effect up to the highest tested concentration of 1,000 ppm. In addition, the proportion of urinary radioactive components was independent of exposure

concentration, except for one component which had a small (less than 10%) increase in proportion to exposure concentration in urine collected at 6 and 24 hours. Two of three metabolites were positively identified as methylthioacetic acid sulfoxide and N-acetyl-S-methylcysteine. The third metabolite was tentatively identified as N-(methylthioacetyl)glycine. All these can be formed from S-methylglutathione. Thus, the conjugation of methyl chloride with glutathione is an important step in the metabolism of methyl chloride.

Conjugation with glutathione is probably not the only route of metabolism. Carbon atoms derived from methyl chloride have been found to be bound to a number of tissues and components that make up the tissues (43, 50). This binding, as shown by Redford-Ellis and Growenlock (47), could result from adduct formation by a chemical reaction of methyl chloride with protein or other cellular constituents. Kornbrust et al. (50), on the other hand, have shown that incorporation of labeled carbon into the actual cellular constituents through single-carbon metabolic pathways is possible. These investigators have shown that the carbon from ^{14}C-labeled methyl chloride can be incorporated into protein, lipids, RNA, and DNA of a variety of rat tissues following a 6-hour exposure to 500 or 1,500 ppm methyl chloride via inhalation. Pretreatment of the animals with methotrexate, which inhibits the one-carbon metabolic pathway, reduced incorporation of the carbon into DNA by 93% and into other constituents by 47 to 65%. Methanol, which

is metabolized to formaldehyde and enters the one-carbon metabolic pathway, also inhibited the incorporation of the carbon from methyl chloride. Additionally, when DNA from treated rats was hydrolyzed and the purine bases separated by column chromatography, the radioactivity eluted with the normal and not the methylated bases. Since methylated bases were not detected, DNA adduct formation may not have occurred. However, the investigators pointed out that the sensitivity of the assay was not sufficient to rule out DNA adduct formation. Adduct formation to protein may have occurred, since pretreatment of animals with cycloheximide, a protein synthesis inhibitor, reduced ^{14}C incorporation into protein by approximately 50% while it inhibited incorporation of ^{3}H-leucine into protein by 75% or more. The higher ^{14}C incorporation suggests that the additional incorporation was the result of adduct formation, possibly through direct alkylation. Thus, the nonextractable radioactivity in tissue protein of rats exposed to ^{14}C-methyl chloride observed by Landry et al. (43) may have occurred through two pathways, incorporation into the protein itself and direct alkylation. Since Landry et al. (43) observed a plateau in the levels of nonextractable radioactivity in kidney tissues between exposure concentrations of 225 and 1,000 ppm, there was probably saturation of one or both of these pathways. The most likely saturable pathway is incorporation of ^{14}C from methyl chloride into the one-carbon pathway.

In order for the carbon of methyl chloride to enter the one-carbon metabolic pathway, it must first be metabolized to the appropriate species, formaldehyde or formate. Heck et al. (51) found a significant increase in liver, testis, and brain levels of formaldehyde from rats exposed via inhalation to 3,000 ppm methyl chloride for 6 hours per day for 4 days. Kornbrust and Bus (52) were unable to find increases in blood or urine levels of formate in rats exposed to up to 10,000 ppm methyl chloride for 3 hours. When methanol, which is metabolized to formate, was given to rats at a dose of 50 mg/kg, increased formate blood levels were found. At a dose of 10 mg/kg, methanol did not increase the formate blood level. When the animals were pretreated with methotrexate or nitrous oxide, both inhibitors of the one-carbon pathway, significant increases in the levels of formate in blood and urine were observed in rats exposed to methyl chloride. The fact that formate levels did not increase under normal conditions at a high concentration of methyl chloride suggests that oxidation to formate is not a rate-limiting step in the metabolism of methyl chloride, even at a high exposure concentration. Since the formate metabolic pathway is saturable, as was seen for methanol at a dose of 50 mg/kg, a rate-limiting saturable step in the metabolism of methyl chloride may be earlier in its biotransformational pathway.

The metabolism of methyl chloride to formaldehyde or formate may occur in part through the microsomal mixed function oxidase

(MFO) system. Dodd et al. (49) investigated whether induction of MFO activity, using the broad spectrum inducer Aroclor 1254, or inhibition of MFO activity, using the classical inhibitor SKF-525A, would have an effect on the reduction of tissue NPSH levels by methyl chloride. Rats were pretreated with the inducer or inhibitor and then exposed to an air concentration of 500 ppm methyl chloride for 6 hours. Neither the inducer nor inhibitor significantly altered the effect of methyl chloride on tissue NPSH levels. Kornbrust and Bus (50) examined the effect of MFO inducers and inhibitors on incorporation of ^{14}C into tissue components of the liver. Rats were pretreated with the inducers Aroclor 1254, 3-methylcholanthrene or phenobarbital, or with the inhibitor SKF-525A, and then exposed to 1,500 ppm methyl chloride for 6 hours. Phenobarbital was the only inducer to have an effect. It caused a significant increase in incorporation of ^{14}C into the lipid- and acid-insoluble portion of the tissue. SKF-525A reduced ^{14}C incorporation into the different tissue fractions examined but the decrease was only significant for the whole homogenate and not for individual tissue components. These investigators also showed that isolated rat liver hepatic microsomes were capable of metabolizing methyl chloride to formaldehyde. This enzymatic reaction required a necessary co-factor for the MFO, NADPH. In addition, inhibitors of MFO activity in vitro, SKF-525A and carbon monoxide, reduced the formation of formaldehyde. Phenobarbital pretreatment of the rats before the liver microsomes were isolated increased the

formation of formaldehyde by a factor of 2.3. The demethylation rate for methyl chloride in this in vitro system was 15 times less than the demethylation rate of benzamphetamine, which suggests that this pathway of metabolism is relatively slow.

Glutathione may be important in the transfer of the carbon in methyl chloride to the one-carbon metabolic pathways. Kornbrust and Bus (53) have shown that glutathione is involved in the formation of the carbon dioxide from methyl chloride. Carbon dioxide accounts for nearly 50% of the recovered radioactivity after 6 hours of exposure to methyl chloride. Inhibitors of the one-carbon metabolic pathway have been shown to reduce expiration of labeled carbon dioxide following exposure to ^{14}C-methyl chloride (50, 52). Additionally, SKF-525A pretreatment significantly reduced expiration of ^{14}C-carbon dioxide from inhaled methyl chloride (53). Kornbrust and Bus (53) showed that pretreatment of rats with diethyl maleate, a compound which depletes tissue NPSH, significantly reduced the expiration of ^{14}C-carbon dioxide from rats exposed to 1,500 ppm methyl chloride via inhalation for 6 hours. Pretreatment of rats with S-methylcysteine also reduced the expiration of ^{14}C-carbon dioxide. These findings suggest that S-methylglutathione is metabolized not only to the urinary metabolites previously discussed, but also to a point where the methyl group is removed and it enters the one-carbon metabolic pathway. Thus, addition of S-methylcysteine would compete with S-methylglutathione

for metabolism and effectively inhibit formation of carbon dioxide from S-methylglutathione.

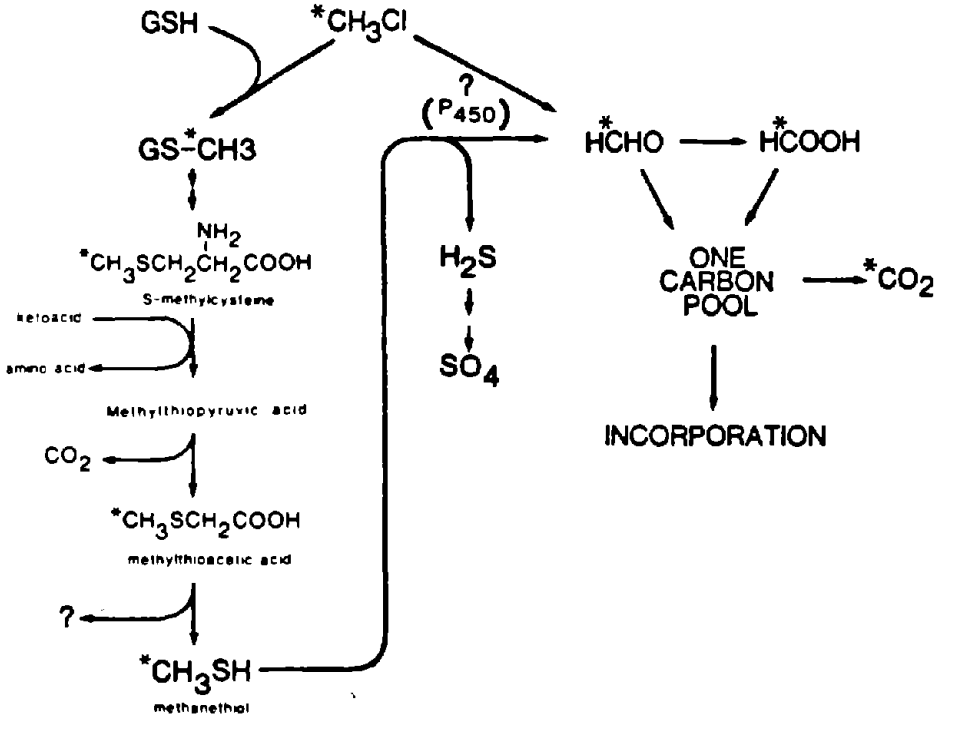
Kornbrust and Bus (53) and Landry et al. (43) have proposed pathways for metabolism of methyl chloride. They are presented in Figures III-2 and III-3.

C. Methyl Bromide

There is very little information on the pharmacokinetics and metabolism of methyl bromide. Andersen et al. (42) studied the uptake of methyl bromide by rats in the same system as was previously described for the investigation of methyl chloride. Unlike the kinetics of uptake reported for methyl chloride, where both a saturable rate process and first order rate process were said to be necessary to describe the uptake rate curve, only a first order rate process was necessary to describe the uptake of methyl bromide over a range of concentrations from 100 to 3,000 ppm. This conclusion appears reasonably convincing, since the original data were presented for methyl bromide. In a followup study, Gargas and Andersen (54) examined the production of bromide ion as measured in the blood. In this study the animals were exposed to constant airborne concentrations of methyl bromide for 2 hours. Blood levels of bromide ion were then determined. Assuming that a steady-state concentration had been reached, the rate of bromide production could be determined. The bromide production for varying exposure concentrations of methyl bromide appeared to follow a first-

FIGURE III-2

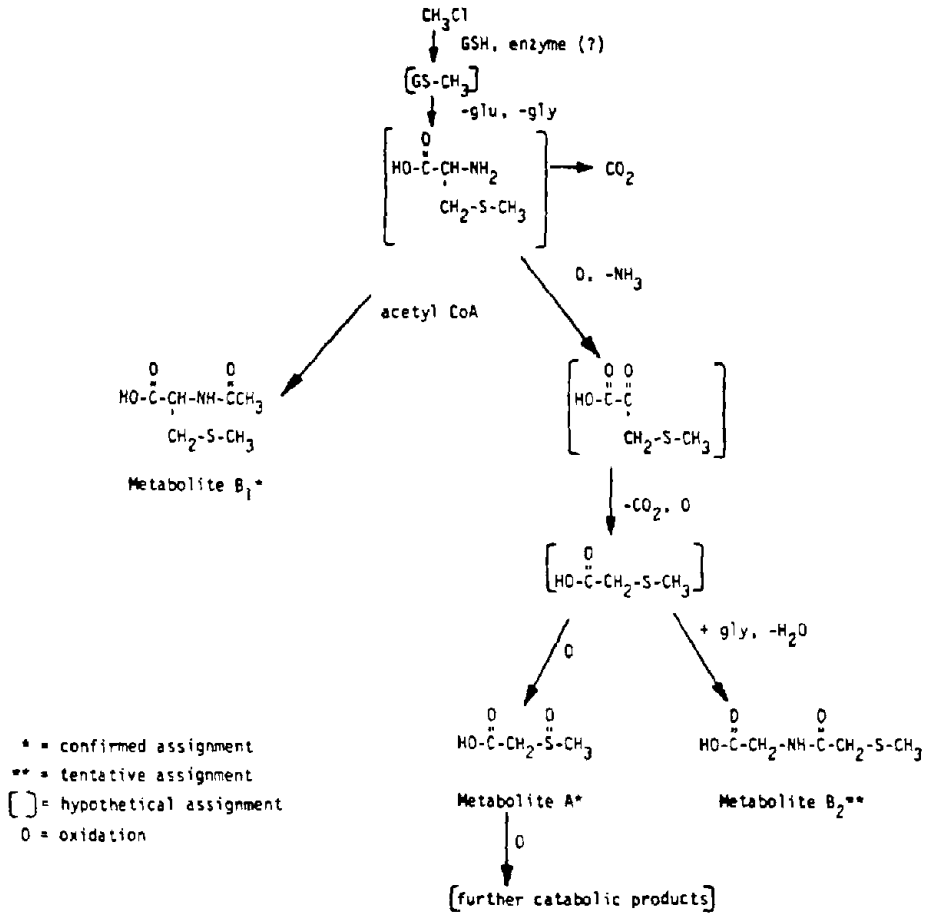
PROPOSED PATHWAY OF METABOLISM FOR METHYL CHLORIDE



SOURCE: Ref. 53, Figure 4

FIGURE III-3

PROPOSED PATHWAY OF METABOLISM FOR METHYL CHLORIDE



SOURCE: Ref. 43, Figure 6

order rate process like that for uptake. The first-order rate constant for uptake was 0.55/kg/hour (or 0.44/kg/hour as reported by Andersen et al. (42) and the constant for bromide production was 0.32/kg/hour.

The first-order rate processes suggest that methyl bromide may be rapidly broken down by a nonenzymatic pathway. However, methyl bromide may also be broken down through an enzymatic pathway that appears to be a first-order rate process if the concentration of methyl bromide is sufficiently low that saturation would not be observed.

Sato et al. (55) reported a study in which rats were exposed to an airborne concentration of 400 ppm of methyl bromide for 4 hours. Maximum blood, liver, kidney, heart, and brain levels of methyl bromide were observed within 1 hour. Following exposure, methyl bromide was quickly eliminated and could not be detected 1-2 hours later. Although Sato et al. reported that metabolism in the liver was relatively rapid, tissue bromide levels did not reach a maximum for 1-3 days following exposure.

The excretion of methyl bromide in expired air was examined in rats following gastric intubation of an "oil" solution or intraperitoneal injection of concentrated vapor (56). Exhaled methyl bromide was thermally decomposed and determined as bromide. The injection of vapor lead to expiration of a large portion, 24-54% of the dose. The proportion expired appeared to be independent of dose over the range of 120 to 180 mg/kg. The expiration was rapid with 95% occurring within the first 30 minutes.

Administration of methyl bromide in an oil solution into the stomach drastically reduced the proportion of dose expired. Animals given 1 dose of 75 mg/kg expired an average 2.5% of the dose, and animals given 100 mg/kg, which was fatal in 5 to 7 hours, expired an average of only 4.1% of the dose. Elimination was complete after 2.5 hours. The remainder of the dose was found as bromide in the animal. After administration of methyl bromide into the stomach in an oil solution, absorption would be slow and would possibly prevent saturation of enzymatic metabolism of the compound.

It is possible that nonenzymatic breakdown of methyl bromide may occur in the blood and tissues. Murray (57) has reported that methyl bromide reacted with nitrogen and sulfur compounds when these compounds were in an aqueous environment. No reaction occurred when water was not present. Lewis (58) showed that methyl bromide will combine with the sulfhydryl groups of isolated enzymes producing a progressive irreversible inhibition.

Djalali-Behzad et al. (13) reported adduct formation with protein and DNA exposed to methyl bromide in vivo and in vitro. These investigators reported that methyl bromide incubated with isolated erythrocytes bound to cysteine and histidine residues of hemoglobin. Methyl bromide methylated histidine in protein and guanine in DNA of isolated mouse spleen cells. It also methylated guanine of isolated DNA. In studies in vivo, methyl bromide methylated the sulfhydryl groups of cysteine in hemoglobin and liver protein, and methylated guanine of

spleen and liver DNA of mice exposed via inhalation. These latter findings are questionable, since the amount of DNA binding reported appears to be below any reasonable detection limit (see discussion in Section II.D.6 above).

A likely major route of metabolism for methyl bromide is enzymatic conjugation with glutathione. Johnson (59) showed that purified glutathione S-alkyltransferase from rat liver was able to catalyze this reaction. The reaction rate was determined for a number of methyl bromide concentrations. From the results presented graphically by Johnson (59), the Michaelis-Menten constant, K_m, of this reaction was calculated by us to be about 2.9 mM. Johnson did not calculate this value, because the loss of methyl bromide from the reaction mixture made it impossible to determine the actual concentrations present. If the concentrations were less than reported, then the K_m value would be smaller than 2.9 mM.

D. Methyl Iodide

As with methyl bromide, there is very little information on the pharmacokinetics and metabolism of methyl iodide. There is one series of reports by investigators who examined the retention and metabolism of methyl iodide in human subjects (60, 61, 62). In these studies, subjects inhaled air containing ^{132}I-labeled methyl iodide (concentration not indicated) for 5 minutes. The isotope iodine-132 is a gamma emitter that can be detected while still in the body by external gamma detectors. Expired air passed through a charcoal trap that retained

the expired methyl iodide, which was then determined by its radioactivity. In these studies, 18 subjects were used. Retention of methyl iodide by these subjects averaged 72% and varied from 53 to 92%. The percentage of the compound retained appeared to be inversely correlated with the respiration rate. This was found when retention and respiration rate were plotted for the 18 subjects and when respiration rates were varied for two subjects. It was reported that there was no exhalation of methyl iodide following the exposure period. However, it was also reported that methyl iodide could be detected in expired air for at least 10 seconds after one inspiration. If all inspired methyl iodide had been retained, that would mean absorption had occurred in all areas of the pulmonary tract. The partial retention found in these subjects indicates that methyl iodide was absorbed from alveolar air and not from dead space air. However, calculations indicated less than 100% retention from alveolar air. When the absorption of methyl iodide from the lung was followed by an outside gamma detector, it was found that the absorption half-time was 2.2 to 5 seconds. This explains why a greater respiratory rate decreases retention, since the amount of the compound that can be absorbed is reduced when the length of time the compound remains in the lung is shortened.

The finding that little, if any, absorbed methyl iodide was expired suggests that it was quickly removed from the blood. These investigators measured the thyroid uptake and urinary excretion of the iodine from inhaled methyl iodide. They found

exponential uptake and excretion curves. These curves compared very closely to the uptake and excretion curves generated when a subject ingested ^{132}I-sodium iodide. The similarity in the curves suggests that methyl iodide is broken down very quickly, which is in agreement with the observed lack of excretion in expired air.

Johnson (59, 63) examined the metabolism of methyl iodide in rats. The rats were given the compound orally and the amount of exhaled methyl iodide was determined. At the LD_{50} dose of 76 mg/kg, only 1% of the dose was exhaled, which is similar to the finding by Miller and Haggard (56) for methyl bromide; in the latter study rats orally dosed with 75 to 100 mg methyl bromide exhaled only 2.5 to 4.1% of the administered dose. A similar study was not found on methyl chloride, but other animal and human studies (43, 46) suggest that a larger percentage of the administered dose would be exhaled, than found for methyl iodide or methyl bromide.

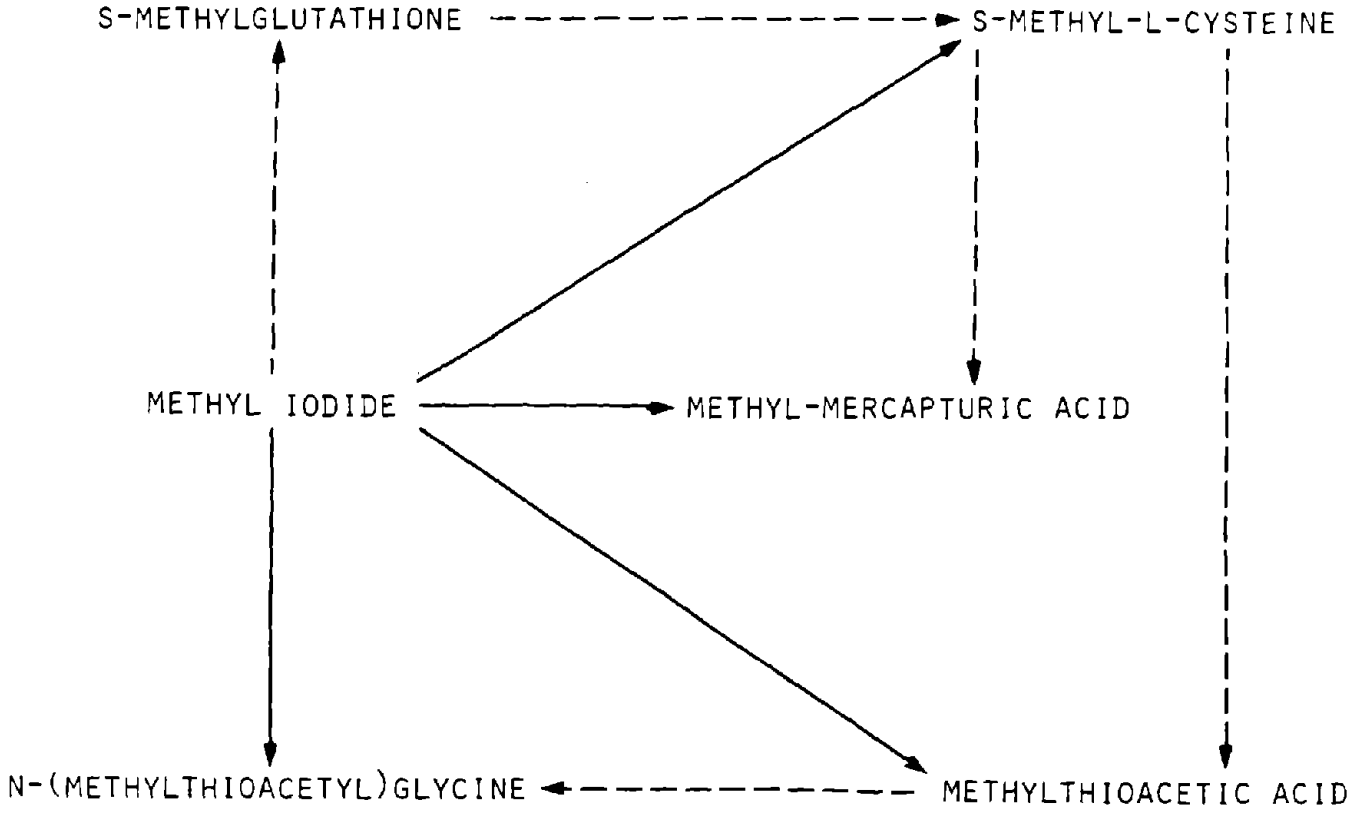
Johnson (56) reported that oral administration of 50-100 mg/kg methyl iodide reduced NPSH levels in the rat liver and kidney. The same doses also reduced the level in brain, but the number of animals examined was small. In deproteinized extracts of livers from treated rats, Johnson (63) isolated a compound with chromatographic properties which were the same as S-methylglutathione. S-methylglutathione as well as traces of S-methylcysteine were found in the bile of a treated rat. The formation of S-methylglutathione was shown to be enzymatically catalyzed

(59). The loss of methyl iodide was measured in liver homogenates. Methyl iodide was lost from the homogenates at a rate of 1.7 μmoles/minute/gram tissue. When glutathione was not added or the homogenate was initially heated to 70°C for 4 minutes, loss of methyl iodide was not detectable. Activity was also found in kidney and adrenal but not in blood or other tissues. The enzyme responsible for this reaction was glutathione S-alkyltransferase. Johnson (59) isolated this enzyme from rat liver and determined the K_m, 0.37 mM, of the enzymatic reaction for methyl iodide as the substrate. This enzyme activity was found in the liver and kidney of a variety of species, including human liver.

Johnson (63) found that S-methylglutathione was not degraded futher in liver homogenates but was degraded in kidney homogenates to S-methylcysteine, glycine, and glutamic acid. No other metabolites were identified, although some were found. Barnsley and Young (64) did identify other urinary metabolites. They injected rats subcutaneously with a 10% solution of methyl iodide in arachis oil giving each rat about 50 mg methyl iodide/kg. Urine collected in the first 24 hours contained four metabolites. Urine collected in the following 24 hours did not contain these metabolites. The four metabolites identified by paper chromatography were S-methyl-L-cysteine, N-(methylthioacetyl)glycine, methyl-mercapturic acid, and methylthioacetic acid. These investigators presented a possible metabolic scheme based on their findings, Figure III-4. This scheme is essentially the

FIGURE III-4

PROPOSED PATHWAY OF METABOLISM FOR METHYL IODIDE



Conversions demonstrated in reference 57 are shown by continuous lines, and possible metabolic pathways are shown by broken lines.

SOURCE: Ref. 64, Scheme 1

same as that proposed by Landry et al. (43) for methyl chloride, Figure III-3, although it is not as detailed. Since methyl bromide is enzymatically conjugated to glutathione to form S-methylglutathione, it should also have a similar metabolic pathway. No information was found on whether methyl iodide was metabolized by the mixed function oxidases as proposed for methyl chloride (53).

E. Conclusions and Summary

Review of data on the pharmacokinetics and metabolism of the monohalomethanes shows that methyl chloride is the best studied of the three compounds. However, the information on this compound is limited. The two pharmacokinetic studies on methyl chloride differ on the kinetics involved. One study (42) suggested that there may be both saturable and first order kinetics, while the other study (43) indicated that no saturable kinetics were involved with methyl chloride at least up to an exposure concentration of 1,000 ppm. The latter study appears more convincing because the former study used an indirect method and was inadequately reported. Even though pharmacokinetic modeling did not show saturable kinetics in this latter study, one aspect of the study did indicate that there may be saturation of some process in the biological handling of methyl chloride, since nonextractable kidney levels of radioactivity did not increase in proportion to the exposure level. This effect may result from saturation of an enzymatic process. Studies have shown that methyl chloride can be enzymatically metabolized

by at least two pathways, enzymatic conjugation with glutathione and metabolism via the mixed function oxidases. Both pathways can apparently lead to the incorporation of the carbon atom from methyl chloride into the one-carbon metabolic pathways. In this way, carbon from methyl chloride may be excreted as expired carbon dioxide or may be incorporated into cellular constituents. Data on metabolism of methyl chloride suggest that the one-carbon pathway is not saturated even at high exposure levels (52). Additionally, conjugation with glutathione appears to be rapid, although at high concentrations tissue levels of NPSH can be depleted and this might contribute to saturable kinetics. However, a more likely saturable (or at least rate-limiting) step is metabolism by the mixed function oxidases. An in vitro study (49) showed that demethylation of methyl chloride was 15 times slower than demethylation of another substrate, benzamphetamine. The importance of these possible saturable pathways is not known, and they may not significantly influence tissue levels of methyl chloride.

First-order kinetics may result from three processes, all of which probably occur with methyl chloride. The first process is tissue redistribution from richly perfused tissue to poorly perfused tissue. Secondly, methyl chloride chemically alkylates cellular constituents (48, 50), although this does not appear to be very important (50). The third process is an apparent first-order enzymatic reaction which occurs when the substrate concentration is below the Michaelis-Menten constant,

K_m, of the enzymatic reaction. A proportional increase of tissue nonvolatile metabolites of methyl chloride with exposure level suggests that such a reaction occurs (43).

In the context of quantitative risk assessment, the most important question is whether a saturable process will result in a nonlinear relationship between external exposure concentrations and internal doses at the target tissues. An indication that such an effect is occurring would be a disproportionate increase in the proportion of DNA adducts formed by chemical alkylation. This adduct formation could be detected by measuring nonextractable radioactivity following exposure to labeled methyl chloride. Landry et al. (43), however, did not find a disproportionate increase in nonextractable radioactivity in rats exposed to increasing air concentrations of methyl chloride. Instead, they found that the levels of nonextractable radioactivity plateaued, at least in the kidney, which is the target tissue in mice. This plateauing suggests either that the enzymatic formation of a reactive metabolite is saturable, or that the metabolism of methyl chloride to a point where it enters the one-carbon pathway is saturated. In either case, this finding indicates that any saturable process in the metabolism of methyl chloride would decrease rather than increase its carcinogenic potential at high doses. However, this information is available only for rats and dogs and does not necessarily apply to mice or humans.

A potentially important role for pharmacokinetic and metabolic information in quantitative risk assessment is to guide the selection of appropriate scaling factors for dose between experimental animals and humans. For example, if humans were found to metabolize a compound more slowly and to retain it for longer than experimental animals, this would suggest that humans would be at higher risk than animals exposed to the same concentration. Unfortunately, the data on metabolism and pharmacokinetics of methyl chloride in humans are very limited and are not suitable for use in such comparisons. The human data indicate that methyl chloride is metabolized relatively rapidly compared to other chlorinated methanes, but that it is retained for long enough (1 hour or more) to reach potential target tissues.

The data on methyl iodide and methyl bromide are also inadequate for such comparisons to be made. Pharmacokinetic data on methyl bromide in rats (42) indicate that only first-order kinetics are involved. Since it was also found that formation of free bromide in the blood of rats exposed to methyl bromide was first order with a rate constant that was 50 to 75% of the uptake rate constant, this suggests that methyl bromide is rapidly broken down or metabolized (54). This finding is supported by the fact that little methyl bromide was found to be excreted in expired air after oral intubation of rats (56). Methyl iodide was also found not to be excreted in expired air in a similar animal study and also a human inhalation study

(61, 63). Although methyl bromide has been shown to alkylate protein in vivo (13), the amount of adduct formation could not account for most of the breakdown of methyl bromide. It appears likely that enzymatic metabolism of methyl bromide and methyl iodide account for the rapid breakdown of these compounds. The enzyme glutathione S-alkyltransferase may be able to catalyze the reaction between these compounds and glutathione at a sufficient rate to account for the rapid loss. The Michaelis-Menten constant for this enzyme reaction with methyl iodide as the substrate was found to be 0.37 mM (59), and with methyl bromide as the substrate 2.9 mM, as calculated from data presented by Johnson (59). Steady-state blood levels of methyl chloride in animals exposed to an airborne concentration of 1,000 ppm ranged from 0.064 to 0.081 mM (43). At similar blood and tissue levels of methyl bromide or methyl iodide, enzymatic breakdown by gluatathione S-alkyltransferase would appear to be first-order.

The metabolism of methyl iodide and methyl bromide is probably similar to that of methyl chloride and, in fact, similar metabolites have been identified (43, 64). However, there is insufficient information on relative rates of metabolism and preferred routes. It is not known if reactive metabolites are formed or if adduct formation is by direct alkylation by the parent compounds. If it is by the parent compounds, then methyl chloride may have greater carcinogenic potential than the other monohalomethanes, since it may exist in the body

unaltered for a longer time, allowing it to reach sensitive organs like the kidney. On the other hand, if a metabolite is responsible for a carcinogenic effect, the other compounds may have greater carcinogenic potential. Also, the greater reactivity of methyl bromide and methyl iodide may simply indicate that they are more likely to act at the sites of first contact with the body (e.g., the lung), while methyl chloride is more likely to act at distant sites (e.g., the kidney). There is not enough information to draw definitive conclusions on these issues.

In conclusion, the data on pharmacokinetics and metabolism of monohalomethanes are consistent with first-order kinetics and a linear relationship between whole-body exposure concentrations and doses to the target tissues. Although methyl bromide and methyl iodide are probably metabolized more rapidly than methyl chloride, there is no reason to infer that this indicates a greater or lesser carcinogenic potential than would be indicated by their biologic activity. Human data are insufficient to be useful in guiding the choice of interspecies scaling factors for dose.

IV. METHODOLOGIES FOR CARCINOGENIC RISK ASSESSMENT

A. Introduction

In this chapter of the report, we summarize the methodologies available for carcinogenic risk assessment and discuss a number of issues arising in the use of these methodologies. In recent years, models and procedures for low-dose carcinogenic risk assessment have been discussed in detail by many expert scientific committees and government regulatory agencies (65-74). This chapter of the report extends and updates these published discussions by incorporating references to recent critiques and proposals, and by focusing attention on some practical problems that arise in the application of these models and procedures. The chapter falls into two parts. The first is concerned with the use of animal data in carcinogenic risk assessment and the selection of a unit of dosage that will be equivalent in different species. The second is concerned with the choice of an appropriate mathematical model of the dose-response relationship.

B. Issues Involved in Dose Adjustment and Interspecies Scaling

1. Introduction

For quantitative estimates of carcinogenic risks to humans to be calculated, it is necessary in most cases to use incidence data from laboratory studies, usually on rodents, to fit the data to a dose-response curve and thereby estimate the magnitude of the risks likely to occur at low dose levels. Even when

the carcinogenicity of a substance has been demonstrated in humans, it is frequently not possible to derive exposure estimates accurate enough for use in estimating the parameters of a dose-response curve. Also, even when adequate dose-response information is available on human exposure to a substance by one route, it is sometimes necessary to estimate human risks resulting from exposure by another route. Therefore, it is necessary to analyze the relationship of a given dose in certain units for a particular species treated by a specified route and dosing schedule to a dose of the same substance administered differently.

A number of difficult issues arise in this process. First, doses may be expressed in a number of different units (e.g., quantity administered per unit body-weight, quantity administered per unit body-surface area, concentration in ambient air, or concentration in the diet). The relationships between these measures of dose for a given species are known to depend on body size, food consumption, metabolic rate, and other scaling factors. Hence the relationships between the measures of dose vary considerably between species. It is not clear which measure of dose should be assumed to be equivalent in terms of toxicological effect in different species. Second, humans live much longer than experimental animals, and it is not clear how this should be taken into account in interspecies extrapolation. At one extreme, it could be assumed that dose rates in mg/kg/day would produce equivalent toxic effects in different species;

at another extreme, it could be assumed that dose rates in mg/kg/lifetime would be equivalent. Third, the relationship between the dose administered to an animal and the quantity reaching target organs and tissues depends on metabolic and pharmacokinetic factors that control the absorption, retention, and distribution of the chemical in the body. These factors vary considerably between species and may also depend strongly on other factors such as the route of administration.

2. Use of Animal Data to Estimate Human Risk and a Unit of Equivalence for Expressing Dose

To justify the use of the results of carcinogenicity testing in animals to make quantitative estimates of cancer risks in humans, a sequence of relationships must be demonstrated.

First, it is necessary to inquire whether there is a qualitative correspondence between the ability of various substances to cause cancer in different species. Of particular interest are the situations in which humans are one of the species in question, although a consistent pattern across a number of nonhuman, mammalian species offers some assurance that humans are also likely to be susceptible.

Tomatis et al. (75) found that the induction of liver tumors in the mouse was a generally reliable predictor of a carcinogenic response in adequate bioassays with rats and hamsters. Purchase (76) compiled bioassay results on 245 chemicals tested in rats and mice. His sources of information were the Federal Register accounts of the National Cancer Institute Program's studies, the monograph series of the International

Agency on Cancer (77), and U.S. Public Health Service Publication No. 149 (78). After screening the studies for adequacy in terms of histological examination, size, and duration, Purchase found that 16% of the chemicals had given discordant results in rats and mice and that 64% of the 109 substances positive in both rats and mice affected the same organ in both species (76). Tomatis et al. (42) demonstrated that 17 of the 19 specific chemicals then known to cause cancer in humans had also been shown to be animal carcinogens. The exceptions at that time were arsenic and benzene, but subsequently benzene has been found to be carcinogenic in animals (79). In many cases the sites of the induced cancers and their histological types were the same in humans and animals. There is no specific degree of concurrence that would establish the premise that laboratory animals are dependable models for chemical carcinogenesis in humans, but studies such as those cited above have made it a generally accepted principle in the scientific community.

The second stage in establishing the scientific basis for quantitative estimation of human risk of cancer on the basis of animal bioassays is demonstration of a correlation in the magnitude of carcinogenic effects across species. Establishment of such a correlation is made difficult by a number of factors, including the paucity of dose-response information for carcinogens, especially in species other than rats and mice, by variability in the carcinogenic response observed in different

experiments with the same species, and by uncertainty about the appropriate measures of dose for interspecific comparisons.

The specification of dosage requires consideration of three elements: the amount of the substance administered, some measure of the size of the organism receiving the exposure, and a temporal descriptor. There are several possibilities for each of these elements. The most widely used measures for the quantity of the chemical administered are weight (mg), volume (ml), concentration (ppm or mg/m^3), and number of molecules (moles). Weight is the measure most frequently used, but theories of carcinogenesis that hypothesize that the critical reaction(s) is (are) caused by single molecule(s) would most logically lead to the expression of measures of dose in moles. The measures of the size of organism to which the dose could be standardized include weight (kg), surface area (m^2), amount of DNA per cell, and blood volume (ml). Weight and surface area are the only two measures that are frequently used, with weight being the most common in carcinogenicity research. However, if in fact DNA is the target of carcinogenic action a unit measuring it might be most logical. The timing of dosage may be incorporated in terms of average lifetime daily dose, average daily dose during the period of exposure, maximum daily dose, or cumulative lifetime dose. Risk assessors have not demonstrated a clear preference for any one of these measures of timing of exposure, although toxicologists usually report their studies in terms of the amount of substance actually

administered on a given day. The list of examples given here is not exhaustive, but could be combined to yield 64 (4 x 4 x 4) possible definitions of a dosage unit.

The Office of Technology Assessment (OTA) (73) illustrated the range of estimated human risk that would result if extrapolation from rodent data were performed using four different units for dose (Table IV-1). Among these options, the use of mg/kg/day gives the least conservative (lowest) estimate of human risk, while dietary concentration and $mg/m^2/day$ are successively more conservative (i.e., they predict higher relative human risk), and mg/kg/lifetime is the most conservative of the four considered by OTA. Use of mg/m^2/lifetime would give even more conservative estimates of human risk.

TABLE IV-1

RELATIVE HUMAN RISK DEPENDING ON HOW DOSE RATE IS SCALED FROM EXPERIMENTAL ANIMALS TO HUMANS

Experimental Animal	mg/kg bw/day	ppm in diet	$mg/m^2/day$	mg/kg bw/ lifetime
Mouse	1	6	14	40
Rat	1	3	6	35

SOURCE: Reference 73

Empirical data on dose-response relationships in different species have to be analyzed with these uncertainties in mind. Data on end points other than cancer have been used in an effort to illustrate that the magnitudes of physiological variables have a predictable distribution across species. Krasovskii (80), for example, showed that for a large number of mammals there is a linear relationship between the logarithm of body weight (y) and the logarithms of the measures (x) of a wide variety of biological variables (e.g., organ-to-body weight ratios, physiological constants, enzyme activity). These relationships can be expressed as:

$$x = a\ y^b$$

or, in linear form, as:

$$\log x = \log a + b \log y,$$

Unfortunately, different values for the parameters a and b appear to characterize the different biological variables. Therefore, in order to estimate values for humans for any particular physiological variable, data from more than one other mammalian species would be needed for the estimation of b, the power of body weight, and a, the constant of proportionality defining the relationship.

Using the ordinarily prescribed dosages of five anticancer drugs, Pinkel (81) demonstrated that for a variety of nonhuman mammals (rats, mice, hamsters, dogs) and humans of various ages, the effective dosages were virtually equivalent (within a factor of two) when expressed in units of mg/m^2 surface area.

When expressed in units of mg/kg body weight, the dosages varied by factors ranging up to 28-fold.

Freireich et al. (82) used toxicity data on 18 anticancer drugs to investigate the quantitative relationship in response between humans and other mammals. The maximum tolerated dose (MTD) was the measure used for humans, dogs, and monkeys, while the LD_{10} (the dose estimated to be lethal to 10% of treated animals) was the measure for mice and rats. The route of exposure in almost all cases was intraperitoneal or intravenous. These measures of potency were expressed as cumulative doses adjusted to daily doses for 5 days of administration in units of mg/m^2 body surface area. The rationale for using this unit for dose was:

> Calculations based on units of body surface area have no intrinsic merit per se. Very likely some other basis such as surface area of the site of action of the drug, lean body mass, or some fractional power of body weight, possibly related to length or some organ-membrane surface area, would be as appropriate or more appropriate. However, the body surface area has been used to relate many physiologic parameters among species and means of transforming the data are readily available. Further in our clinical studies we routinely use body surface area to adjust drug dose for patients of different size and weight. (82)

In instances where several estimates were available for a given species and drug combination, the average was taken. When the average human MTDs were plotted (on a logarithmic scale) against the average figures for each species, their magnitudes were found to be closely correlated, and the points for each species fell roughly about a line indicating equivalence with

the human dose. When the average LD_{10}s for BDF_1 mice were plotted against the human MTDs in units of mg/kg body weight, the regression line was offset from equivalence by a factor of 12, which corresponded almost exactly to the ratio of the correction factors (kg/m^2) used to convert doses for humans and mice from mg/kg to mg/m^2. This study represents the most extensive documentation of the equivalence of a measure of human response to a toxic chemical to a similar measure in other mammals when the doses are expressed in terms of body surface area.

Using estimates of MTDs for 40 antitumor drugs culled from references 82 and 83, Dixon (84) calculated the correlation of the logarithms of the MTDs in humans versus those of dogs, monkeys, or the more sensitive of these species. The calculations were repeated with the doses expressed first in mg/kg body weight and then in mg/m^2 body surface area. The correlations using either unit were strong (the exact degree of significance was not specified), but the author reported that MTDs were more nearly equivalent when expressed in mg/kg. The regression lines in mg/m^2 were offset from equivalence by approximately a factor of 2. The regression line for prediction of the human MTDs on the basis of the MTDs in the more sensitive species had a slope of 1.0 (exact equivalence) when mg/kg was the unit used.

It should be noted that dogs and monkeys are more similar to humans with respect to both surface area and body weight

than are rats or mice, so that conversion between these units does not result in as large differences in the outcome of regression analyses as it would for a much smaller laboratory rodent and humans. Similarly, data sets over a narrow range of animal size cannot be expected to be as sensitive in distinguishing the unit most appropriate for expression of equivalent doses for the all mammalian species.

A Committee of the National Academy of Sciences (85) compared the carcinogenic potency in humans and animals of six known human carcinogens that had sufficient data for at least rough estimation of human exposure and induced incidence. This selection criterion could theoretically have imposed a bias toward chemicals to which humans are relatively sensitive, thus exaggerating the sensitivity of humans in comparison to laboratory animals. The six substances reviewed were benzidine, chlornaphazine, DES, aflatoxin B_1, vinyl chloride, and cigarette smoke. The doses were expressed in units of mg/kg bw/lifetime. Animal studies following a variety of different protocols were reviewed, and the bioassay showing the greatest sensitivity to each substance was selected. For three of the six substances (benzidine, chlornaphazine, and cigarette smoke), the human response to a given lifetime average dose was found to be similar to the response of the most sensitive animal species exposed to the same lifetime average dose. For the remaining three substances, the animal responses predicted human responses

10 to 500 times higher than had been observed. The conclusion of the report was:

> Thus, as a working hypothesis, in the absence of countervailing evidence for the specific agent in question, it appears reasonable to assume that the lifetime cancer incidence induced by chronic exposure in man can be approximated by the lifetime incidence induced by similar exposure in laboratory animals at the same total dose per body weight. (85)

It could be argued, however, that the difference in average lifespan for humans and rodents means that the use of total lifetime dose would result in the estimates of human risk higher by a factor of approximately 35 than if lifetime average daily dose were used. Which time factor is actually appropriate is not clear, but the tendency of the animal responses to overestimate human sensitivity, and the anticipated bias in the opposite direction introduced by the selection of chemicals to which humans may be particularly sensitive, suggest that average daily dose may be a more appropriate comparative measure of dose.

Mantel and Schneiderman (86) proposed the idea of using doses in units of surface area, not on the grounds of any empirical evidence, but solely on the basis of the assumption that the locus of action of any drug is on some surface. They, however, refrained from making any recommendation:

> Finally, we have written (and behaved?) as if the animal information were directly extendable to man, perhaps on a mg/kg basis, or perhaps on a mg/sq m basis, or perhaps on some other basis. In this paper we have made no attempt to indicate which, if any, of these extrapolation procedures is good (better or best). To us the reason in obvious. No suitable data exist on the quantitative

extrapolation of carcinogenic effects from animal to man. (86)

Crouch and Wilson (87) investigated the comparability of carcinogenic potency estimates using mg/kg/day as the unit of dosage. The maximum likelihood estimate of the dose-response slope parameter β in the one-hit model for the tumor site giving the most extreme result was used as the measure of carcinogenic potency. For each chemical, an estimate of potency was calculated for both sexes of rats and mice. The National Cancer Institute's bioassays in rats and mice for 90 substances provided the sets of data for the comparison of B6C3F1 mice with Osborne-Mendel rats, Fischer 344 rats, and Sprague-Dawley rats, and of males and females of the individual strains. Graphical displays of these comparisons were presented, but the actual estimates of best-fit lines and their significance were not included. In each case, the potencies (expressed in units of response per unit dose in mg/kg body weight) appear quite strongly correlated. However, without a similar analysis after the potencies had been calculated on the basis of mg/m^2 or other dosage units, no assertion could be made that mg/kg provides the optimum expression of equivalent dose.

Using the mathematical methods of the previous study (87), Crouch (88) presented further results on interspecies correlations in carcinogenic potencies based upon data for 187 chemicals tested in rats and mice by the National Cancer Institute's bioassay program. He found that average lifetime dose in mg/kg provided a higher correlation between the estimated potencies

in Osborne-Mendel rats and B6C3F1 mice than did doses based on surface area, whereas the surface area units gave a higher correlation between the potencies for Fischer 344 rats and B6C3F1 mice. This finding does little to resolve the problem surrounding the dosage unit. As mentioned with respect to the Dixon study (84), the animal species considered are so similar in size that results derived for them may not be generally applicable for equating potencies in all mammals. In this study, Crouch (88) also noted that, although the estimates of potency between species were significantly correlated, the observed variability around the regression line representing perfect agreement exceeded by a factor of about 4.5 the variability predicted solely on the basis of binomial error and sample size.

Crouch and Wilson (87) also assembled estimates of carcinogenic potency in mg/kg in humans and in rats or mice for 13 chemicals. The studies yielding the potency estimates were much less uniform in design than the NCI bioassays used in the previous studies, meaning that much more "noise" would be expected to exist that might obscure any relationship. Nevertheless, the human and rodent potencies were found to be correlated, although not as strongly as those of the sexes within a strain or those of mice and rats. Humans were found to be approximately five times more "sensitive" to these substances than rodents, when sensitivity was defined as response per unit increment of dose in mg/kg. However, this result could be due to expression

of the potencies in a nonequivalent unit or more simply to heterogeneity and imprecision in the original data set.

Crump et al. (89), using the information presented in references 85 and 87, looked at the relative potencies of chemicals in mice, rats, and humans, when potencies were expressed in four different units: (1) mg/kg body weight/day, (2) ppm in diet, (3) mg/m^2 surface area/day, and (4) mg/kg body weight/lifetime. Their results indicated that the conversion of mg/kg body weight/lifetime exaggerates human risk when using mouse and rat data. They found that on the average all measures of dose overestimated the potency in humans, but that conversions to mg/kg body weight/day "on average tend to predict the potencies estimated from human data quite well."

Following the suggestion of Mantel and Schneiderman (86), EPA (72) adopted lifetime average mg/surface area/day as the dosage unit it would consider equivalent across species in quantifying human risks for its water quality criteria documents. To convert from doses in mg/kg, surface area would be assumed to be proportional to the two-thirds power of body weight. For exposure by inhalation, EPA (72) assumed that concentrations in ppm or $\mu g/m^3$ would be a toxicologically equivalent measure of dosage in different species. Although the rationale for this choice was not explained in detail, it appears to reflect the idea that the amounts of a chemical inhaled at a given concentration by different species are proportional to their breathing rates, which in turn are proportional to

their metabolic rates. It is known that in different species, metabolic rates are approximately proportional to the two-thirds power of body weight, i.e., to body surface areas (81). Hence, the assumption that effective doses in different species are proportional to body surface area is equivalent to the assumption that effective airborne concentrations are the same for different species.

More recently, Mantel (90) has expressed the opinion that dietary concentrations may be used successfully for cross-species dose equivalency for ingested agents. Such measures would have a rough equivalency on a surface area or airborne concentration basis (73).

To date no study with the explicit objective of determining what unit best expresses equivalence of carcinogenic potency across mammalian species has been conducted. Because of the numerous other factors influencing the comparability of potency estimates (experimental error, route of administration, model in which potency was estimated, dosing schedule, chemical-specific interactions with animals, etc.), the compilation of a data set to determine this definitively would be exceedingly difficult. The possibility also exists that there is in fact no universally equivalent unit of dose. Also, the most extreme difference resulting from the use of mg/kg rather than mg/m^2 or ppm, for instance, would be a factor of 14 for estimation of human risk from mouse data; in view of the overall uncertainty surrounding risk estimates, a factor of this magnitude may

not be of prime importance. Since for so many other reasons estimates of carcinogenic risk to humans cannot be regarded as precise predictors of actual numbers of cancer cases or deaths, it would seem wise to use a particular unit uniformly so that estimated risks for various substances would be comparable for ranking purposes. The unit of mg/kg/day is most commonly used by experimenters, but it may be more appropriate to use ppm or $mg/m^2/day$, since these fall near the middle of the range of the measures that have been proposed (Table IV-1).

3. Routes of Absorption

In performing quantitative risk extrapolations, it is sometimes necessary to use data from a study in one species exposed by a given route to estimate risk to the same or a different species exposed by a different route. For example, it may be necessary to estimate human risk due to dermal exposure to a compound, using data from an epidemiologic study in which human exposure to that compound was via drinking water. Other situations might require, for example, the use of data from a laboratory study in rats exposed via ingestion to estimate carcinogenic risks to humans exposed by inhalation. Thus, it is important to consider what adjustments in dose might be necessary to account for differences in routes of exposure.

In attempting to make adjustments for differences in the route of exposure, one is concerned with how much of the administered dose is absorbed by the organism. Absorption may be defined as "the process by which a toxicant is able to pass

body membranes and enter the bloodstream" (91). The amount of a given compound likely to be absorbed by a given route depends to a large degree on the physical and chemical properties of the compound. Important properties include its molecular size and shape, solubility at the site of absorption, degree of ionization, and relative lipid solubility of ionized and un-ionized forms (92). These and other factors influencing the rate and extent of absorption are discussed below as they relate to the different routes of exposure.

a. Absorption through the gastrointestinal tract

Some of the factors which influence absorption by the gastrointestinal tract are also important factors in absorption by other routes. These factors include surface area and blood flow at the site of absorption, the physical state of the chemical, and the concentration of the chemical at the site of absorption. In addition, because the gastrointestinal epithelium is made up of cells with tight intercellular junctions and small membrane pores, absorption is mostly limited to nonionized (lipid-soluble) compounds which can readily diffuse through the cells by dissolving in the lipid component of the cell membrane (92). For example, "the absorption of a lipid-soluble nonelectrolyte such as ethanol is very rapid and is limited only by surface area and a few other factors" (92). If the chemical is a weak organic acid or base, it will tend to be absorbed by diffusion in the region of the gastrointestinal tract where it exists in its nonionized, lipid-soluble form.

Since gastric juice is acidic and the intestinal contents are nearly neutral, acids will be more rapidly absorbed from the stomach and bases will be more rapidly from the small intestine. However, it is important not to underestimate the ability of the small intestine to absorb weak organic acids. Even if only 1% of the lipid-soluble form of the acid is present in the intestine, the intestine will continuously absorb that amount while equilibrium will be maintained at 1%. And because of the very large surface area of the intestine, due to the villi and microvilli, the overall capacity of the intestine for absorption is very large (91).

Most compounds absorbed by the gastrointestinal tract are absorbed by simple diffusion. Although lipid-soluble substances are absorbed by simple diffusion more rapidly and extensively than nonlipid-soluble substances, the latter may also be absorbed to some degree. Apparently referring to human exposure, Klaassen (91) reported that "upon oral ingestion about 10 percent of lead is absorbed, 4 percent of manganese, 1.5 percent of cadmium, and 1 percent of chromium." The mechanisms by which some lipid-insoluble substances are absorbed is not understood. For example, on the basis of the pH-partition hypothesis, one would not expect pralidoxime (2-PAM), a fully ionized quaternary ammonium compound, to be absorbed to an appreciable extent. Yet, this compound is almost completely absorbed from the gastrointestinal tract (91). Relatively large particles have also been shown to be absorbed by the

gastrointestinal epithelium, possibly by the process of pinocytosis which has been shown to be more active in the newborn than the adult. Some foreign compounds are absorbed by the active transport systems used by mammals for the absorption of food and electrolytes. For example, 5-fluoracil is absorbed by the pyrimidine transport system and thallium may be absorbed by the system that normally transports iron (91).

Absorption may be modified by a variety of other factors. As reported by NAS (93), "The presence of bacteria in the gastrointestinal tract can affect absorption indirectly. Bacteria can convert the original substance into one that is more or less absorbable, and thus alter the apparent toxicity of a chemical or they may convert a nontoxic substance into a toxic one." Compounds can also be altered by the action of the acid conditions of the stomach and the enzymes present in the gastrointestinal tract (91). Alteration of gastric emptying time or gastrointestinal motility can also affect absorption (91, 92). Certain metals have been shown to alter the absorption of certain other metals. Milk has been found to increase absorption of lead, and starvation was found to enhance the absorption of dieldrin (91).

b. Absorption through the lung

The alveolar region is the area of the lung where inhaled substances are readily absorbed. The surface area is large and blood flow to the lung is high and in close proximity to the alveolar air (91). Thus, substances present in the alveolar

air can be absorbed into the bloodstream at an extremely rapid rate.

Substances inhaled as gases are readily absorbed by the lungs. Gas reaches the alveoli and immediately equilibrates with the blood passing through the pulmonary capillary bed.

> The concentration of the gas in the blood as it leaves the lung is dependent on the solubility of the gas in the blood, where solubility is defined as the ratio of the concentration of dissolved gas in fluid (blood) to the concentration in the gas phase at equilibrium....The rate of absorption of gases is variable and dependent on the toxicant's blood:gas solubility. If the gas has a very low solubility, the rate of transfer is highly dependent on blood flow through the lung (perfusion limited) whereas for gases with a high solubility, it is highly dependent on the rate and depth of respiration. (91)

When the substance is in the form of an aerosol, i.e., solid or liquid particles suspended in air, particle size is the decisive factor for determining the degree of retention or absorption of the inhaled substance. Large particles may deposit in the nose, trachea, or bronchi and then be transported by cilia and mucous to the throat where they are swallowed. Absorption may then take place from the gastrointestinal tract. Smaller particles penetrate deeper into the respiratory system and deposit in the smaller bronchi and on the alveolar epithelium (94). Liquid particles, if lipid-soluble, will readily pass the alveolar cell membranes by passive diffusion (91). Solid particles that reach the alveoli are removed or absorbed by mechanisms that are not as well defined as those for removal of particles from the tracheobronchial tree. In general, removal of particles from the alveolar region is a slow process.

Particles may be removed from the fluid layer of the alveoli by aspiration onto the mucociliary escalator of the tracheobronchial region and be swallowed. The particles can also be removed from the alveoli by phagocytosis; the phagocytized particles can then migrate to the distal end of the mucociliary escalator. As a third route of removal, the particles can be removed via the lymphatic system. In general, removal of particulates from the alveoli is relatively inefficient. Compounds which are the least soluble in lung fluids are removed more slowly than the soluble compounds, suggesting that removal is due to dissolution and vascular removal. Some particles may remain in the alveolus indefinitely, forming an alveolar dust plaque or nodule (91).

c. Absorption through the skin

Most chemicals that are absorbed through the skin enter through the epidermal cells rather than through the cells of the sweat glands, sebaceous glands, or through the hair follicles. In order to be absorbed, the chemical must pass through several layers of cells before reaching the systemic circulation. "In contrast, when toxicants are absorbed by the lung and gastrointestinal tract, the chemical may pass through only two cells" (91).

The first stage of absorption involves passive diffusion across the stratum corneum layer of the epidermis. It is postulated that polar substances diffuse through the outer surface of protein filaments of the hydrated stratum corneum and that

nonpolar molecules dissolve in and diffuse through the nonaqueous lipid matrix between the protein filaments. The rate of diffusion of nonpolar chemicals depends on their lipid solubility and is inversely related to molecular weight (91).

The human stratum corneum varies in permeability in different regions of the body due to differences in thickness and diffusivity. Frenkel and Brody (95) showed that large variability in rates of absorption exists depending on the site of application. Absorption variability between sites was over 10-fold for parathion (96) and 40-fold for hydrocortisone (97).

The second stage of absorption is diffusion through the dermis, which contains a porous, nonselective, watery diffusion medium. Chemicals move through this region by simple diffusion which depends on effective blood flow, interstitial fluid movement, lymphatics, and possibly other factors (91).

Absorption of substances through the skin can be altered by a variety of factors. Removal or abrasion of the stratum corneum increases permeability of the epidermis for all types of molecules, large, small, lipid-soluble and water soluble. Water also increases permeability of the stratum corneum. Solvents such as dimethyl sulfoxide (DMSO) can also facilitate absorption (91).

Species differences in skin permeability have been shown. The skin of the cat is less permeable than that of man, the skin of the rat and rabbit is more permeable, and the skin

of guinea pigs, pigs, and monkeys is similar in permeability to that of man (91).

d. Absorption by other routes

The intravenous route of administration introduces the chemical directly into the bloodstream, thereby eliminating the process of absorption. Introduction of a chemical by the intraperitoneal route results in rapid absorption because of the large surface area and rich blood supply of the peritoneal cavity. Substances administered intraperitoneally are absorbed primarily through the portal circulation and thus pass through the liver before reaching other organs. Compounds administered intramuscularly and subcutaneously are usually absorbed more slowly. The rate of absorption by these two routes can be changed by altering the blood flow to the area. Epinephrine, for example, causes vasoconstriction and would thereby decrease the rate of absorption. Also, substances are absorbed more rapidly from solutions than from suspensions.

In some cases the toxicity of a chemical may be dependent on the route of administration. Because a compound injected intraperitoneally will reach the liver via the portal circulation before it reaches the general circulation, it may be completely metabolized or extracted by the liver and excreted into the bile, never gaining access to the rest of the animal. For example, the drugs propanolol and lidocaine are efficiently extracted during their first pass through the liver. Thus if a compound with selective toxicity for an organ other than

the liver and gastrointestinal tract were extracted this way, one would expect it to be less toxic when administered intraperitoneally than if administered by intramuscular or subcutaneous injection. Compounds not metabolized by the liver or excreted into the bile would not be expected to show markedly different toxicities when administered by these three routes unless other factors, such as differences in the rate of absorption, were involved. Thus, preliminary information about the metabolism and excretion of a compound could be obtained by comparing its toxicity by various routes (91).

4. Metabolism and Pharmacokinetics

The dose or amount of compound available internally to cause an effect may be drastically different from the administered dose because of barriers to absorption as discussed above. Further factors can affect the actual amount of compound that is present at a site of action within the body. The pharmacokinetics of a compound involve the distribution, metabolism, and excretion as well as the absorption of the compound. The differences between species, as well as between strains within a species, in the pharmacokinetics of a compound may cause large differences in the ultimate toxic effect between the species or strains. It is thus highly desirable to know the pharmacokinetic parameters of a compound in both experimental animals and man for extrapolation of risk.

Stara et al. (98) discussed the usefulness of pharmacokinetic data to make inter- and intraspecies extrapolation. They stated:

> Distribution data are of great importance in determining target organs or tissues. They may provide supportive evidence for the validity of species-to-species extrapolation. For instance, if an agent has been shown to have similar distribution in both experimental animals and humans, the toxicity data can be extrapolated to humans with much greater confidence (Stara and Kello 1979) [99]. Determination of the metabolic pathway and eventual fate of the test chemical is important in assessing a potential additional health impact of chemical compounds since metabolites may exert a greater toxic effect than the original agent (Gehring et al. 1976) [100]. Finally, the knowledge of the rate of excretion and the resulting biological half-life may also have a bearing on the biological effects of the agent. (98)

Gehring and Rao (101) also indicated the importance of pharmacokinetics in risk extrapolation and stated:

> Hence to assess the hazard of a chemical to man as well as other species, it is essential to elucidate the kinetics for its absorption, distribution, biotransformation, and ultimate excretion, that is, its pharmacokinetics. Only with acquisition of such information can interspecies, intraspecies, and high dose to low dose extrapolations of potential toxicity be made definitively. (101)

They presented an example from a study (102) which shows how pharmacokinetic data can help to explain species and age difference in toxicity. The clearance from blood plasma of 2,4-dinitrophenol (DNP), which has cataractogenic activity in the duckling and immature rabbit but not the mature rabbit, was followed in these animals. The toxic effect was correlated with a slower clearance rate in susceptible animals. Also higher concentrations of DNP in the aqueous humor, vitreous humor, and lens were associated with a slower clearance rate. Further mathematical analysis of the data indicated that the mature rabbit had a more substantial barrier to the movement of DNP from

the plasma to the aqueous humor than the immature rabbit or duckling. This study shows that both the rates of distribution and excretion of a chemical compound can influence its relative toxicity between species and within species. Comparison of excretion rates of some chlorinated hydrocarbons (e.g., DDT and dieldrin) between the laboratory rat, larger animals, and man have shown over a tenfold difference. Man appears to retain some chlorinated hydrocarbons longer than other mammalian species (103).

Wong and Hsieh (104) reported on a pharmacokinetic study on aflatoxin B_1 in mice, rats, and monkeys. They found that the species more sensitive to acute toxic effects of aflatoxin B_1 showed higher volumes of distribution, higher equilibrium transfer coefficients between the blood and tissue, higher levels of aflatoxins in the liver (the target organ) and plasma, and longer biological half-lives in plasma.

Most chemical compounds are metabolized by a process which usually detoxifies the compound and allows it to be more rapidly excreted. Many chemical carcinogens, however, have been shown to be metabolically activated to their ultimate carcinogenic form. The route of metabolism of a compound can vary between animal species. Williams (105) discussed variations in metabolic pathways found between species. Although all species showed the same general pattern of metabolism of foreign compounds, he stated:

> If any foreign compound (or xenobiotic) is administered to more than one species, although one can

> now predict the pathways of xenobiotic metabolism, it is almost certain that species differences in the amounts of predicted metabolites formed and excreted will be found and in some cases gross differences in the actual routes of metabolism will be found. (105)

Numerous reports have indicated that the relative toxicity or carcinogenicity of compounds between species is related to the formation of a reactive compound which interacts with the macromolecules of the target cell. Schumann et al. (106) showed that perchloroethylene was metabolized and bound more readily in the mouse liver than the rat liver and suggested that the higher degree of binding was related to the carcinogenic effect found in the mouse and not in the rat. Miller et al. (107) found that the mouse but not the guinea pig would metabolize 2-acetylaminofluorene (2-AAF) to N-hydroxy-AAF. N-hydroxy-AAF was shown to cause tumors in both mice and guinea pigs while 2-AAF produced tumors only in mice. Thus, the inability of the guinea pig to metabolize 2-AAF to the proximate carcinogen N-hydroxy-AAF makes the guinea pig insensitive to the potential carcinogenic effect of 2-AAF.

The pharmacokinetics of a compound within an animal can be described by a number of different mathematical models. Depending on the degree of detail that is required in the predictions of the concentrations of the compound in tissues, the body of the animal may be modelled as consisting of one, two, or three "compartments." Still more complex models may be required to describe the pharmacokinetics of carcinogenic chemicals that require metabolic activation to exert their

carcinogenic effect. For example, Gehring and Blau (108) presented a complicated model defined by eight differential equations. This scheme was developed for a hypothetical chemical carcinogen which requires activation to a reactive electrophilic metabolite and subsequent irreversible, covalent reaction with a genetic receptor. Gehring and Blau stated:

> Exact solutions of these differential equations are impossible. However, if values for the various constants are obtained and the dose (C_o) is known, the system of equations can be numerically integrated using a computer. Thus, values for each parameter as a function of time following exposure can be obtained as well as, at infinite time, the amount of each of the end products formed. (108)

These models are useful for obtaining a more precise estimate of the actual exposure of the target tissue to a carcinogen. The simplest model can give an estimate of the body burden or tissue concentration of the chemical over time. The more complex model can give an estimate of the amount of interaction between the ultimate carcinogen and the proposed receptor, in this case the DNA of the cell. To use these models, data must be obtained empirically from the species under investigation. The complexity of the studies needed to determine these data is dependent on the complexity of the model under consideration.

Gehring and Blau (108) also discussed the possible effect of conducting studies at high dose levels on the pharmacokinetics of a chemical. They stated:

> As long as pharmacokinetics of a chemical remain linear, any increase in dose will result in an equivalent increase in the concentration of the chemical in tissue at any point in time. However, many metabolic and excretory processes are saturable, and

> as doses of chemicals begin to saturate or overwhelm these processes, it may be expected that there will be a disproportionate increase in the concentration in tissues and consequently, toxicity (see Gehring et al., 1976) [91b]. For these processes, nonlinear pharmacokinetics apply which can be described by the Michaelis-Menten equation.
>
> $$-\frac{dC}{dt} = \frac{V_m C}{K_m + C}$$
>
> In this equation dC/dt is the rate of change in the concentration of the chemical at time t, C is the concentration of chemical at time t, V_m is the maximum rate of the process and K_m, the Michaelis constant, is equal to the concentration of the chemical at which the rate of the process is equal to one-half V_m. (108)

According to this model, when the chemical concentration is much smaller than K_m, the change in concentration of the chemical over time is proportional to the chemical concentration. When the chemical concentration is much larger than K_m, the change in its concentration is dependent solely on the parameter V_m. According to this theory, carcinogenicity studies conducted at high dose levels, where nonlinear pharmacokinetics would be in effect because of saturable metabolic and excretionary processes, may not be accurately extrapolated to lower dose levels using only the administered dose in the dose-response extrapolation.

Gehring et al. (109) used tumor incidence data from a vinyl chloride inhalation carcinogenicity study in rats by Maltoni and Lefemine (110) and metabolic data they generated to examine the dose-response relationship. They found that the metabolism of vinyl chloride was not linearly proportional to the dose administered and could be fitted to the model des-

cribed above. A good linear fit over the entire dose range tested was found by plotting probit response versus log of the amount of vinyl chloride metabolized in four hours. There was not a good linear fit when the probit response was plotted against administered dose. They suggested that the amount of metabolized vinyl chloride was a better measure of dose than administered dose. These data suggest saturation of the animals' capacity to detoxify the carcinogenic metabolite.

Anderson et al. (111) used the data from the paper of Gehring et al. (108) and calculated low-dose risk estimates using two different models, the probit and multistage. The estimates derived from the probit model differed considerably when the pharmacokinetic adjustments were made and when only the administered dose was used. In the multistage model, the two data sets produced results that differed much less. They found that the bioassay data for vinyl chloride could be fitted to both the probit and multistage models when pharmacokinetic data were used. However, because the fitted models diverged in the low-dose range, they stated:

> It is therefore important to realize that even though the incorporation of the pharmacokinetics into the low-dose estimation process better reflects reality, it does not help in resolving the issue of which underlying model to use for the low-dose extrapolation, e.g., probit versus multistage. (111)

Thus, at least in this case, the use of pharmacokinetic data tended to increase the range of risk estimates that might be derived from the empirical data (111).

To use pharmacokinetic data in risk extrapolation between species, the pharmacokinetic parameters must be known for both species. It is unlikely that the pharmacokinetics of most industrially used compounds can be determined for humans and the costs of extensive animal studies may be prohibitive. Thus, although it is desirable to utilize pharmacokinetic data wherever they are available, their usefulness in practice is likely to be limited.

5. Study Duration and Exposure Duration

Druckrey and Kupfmuller (112) found that the latency period for tumor formation induced by a chemical carcinogen, in this case p-dimethyl-aminoazobenzene, was inversely related to the daily dose of the carcinogen. A similar relationship has been found with other carcinogens, such as aromatic hydrocarbons in mouse skin (113), diethylnitrosoamine (114), and ultraviolet light (115). Druckrey found that the relationship between dosage and tumor induction time could be expressed by the equation:

$$dt^n = \text{constant},$$

where d is the dosage, t is the tumor induction time (usually the induction time for 50% of the tumors), and n is a factor specific for each carcinogen which Druckrey (116) found to vary between 1.1 and 6.5 for several carcinogens he studied. However, there is some evidence that this relationship may result from the use of high doses which initiate a number of cancers in each animal, so that what is observed is a change

in the time of appearance of the first tumor, rather than a true change in latent period (71).

Another factor which complicates the use of animal bioassay data for quantitative risk assessment is the rapid increase in tumor incidence with increasing age of the exposed animals. Dosing frequency and duration, as well as duration of observation, may have an effect on the tumor incidence realized and thus on the results of a risk assessment based on the study. Studies which are terminated earlier than the full lifetime of the animal being studied may underestimate the actual lifetime incidence of tumors induced by the compound. Many oncogenicity studies using mice are terminated after 78-90 weeks. Littlefield et al. (117) reported on a large study examining the carcinogenicity of 2-AAF in mice. They found that if the study had been terminated after 18 months, the low incidence of liver cancer at that time might have caused the effect to be overlooked or to have led to the identification of 2-AAF as only a weak liver carcinogen. By extending the study to 33 months, the incidence of liver cancer was greatly increased. The risk associated with 2-AAF would have been underestimated based on the liver tumor incidence if an 18-month bioassay had been performed. Unfortunately the information obtained from a standard bioassay with only terminal sacrifice and intercurrent deaths is usually insufficient to determine the tumor incidence-time relationship.

Crump et al. (89) proposed an adjustment to account for premature termination of an experiment. This involves a downward adjustment to the dose rates to compensate for the study's failure to detect tumors that would appear in old age. The adjustment is made according to the equation:

$$D = d(T_e/T_n)^4$$

where d is the experimental dose rate, T_e is the average age of the experimental animals, and T_n is the average natural life span of the animals. This adjustment was suggested because for many human cancers, the age-specific incidence increases at a rate proportional to the k'th power of age. Doll (118) observed that k appears specific for the type of tumor and varies between 4 and 6.

In a lifetime study with rats, Lijinsky et al. (119) found that the incidence of esophageal carcinoma increased with increased duration of exposure to nitrosodiethylamine. This was also true for the incidence of tumors (carcinomas and papillomas) in the upper gastrointestinal tract. The duration of exposure was 30, 60, and 104 weeks in a study with observation continuing up to 135 weeks. Formation of liver carcinomas also showed a positive trend with increasing exposure duration.

Burns and Albert (120) described a model which covers the additivity of multiple doses of a carcinogen on tumor formation. The basis of the model is the idea that a carcinogen may have two distinct effects on a cell, first causing trans-

formation of the cell, and later accelerating the progression of the transformed cell towards cancer. This latter effect on acceleration is considered dependent on the amount of carcinogen present. It was assumed the probability of tumor formation per unit time was a function of both time and dose and the expression for the relationship between these two parameters at the time of 50% tumor incidence is:

$$dt^{(r+1)/m} = \text{constant},$$

where m and r are the powers of dose and time, respectively, in the instantaneous hazard function. This is the same as Druckrey's equation when $(r+1)/m = n$. In order to estimate tumor incidence when the duration of exposure is less than the lifetime of the animal, the effective dose rate, D, is calculated by the equation:

$$D = (t_0/t_{50})d$$

where t_0 is the time at which dosing was stopped, t_{50} is the time for 50% tumor incidence to occur with continuous dosing, and d is the dosage. Burns and Albert (120) applied this model to liver cancer induced by 2-AAF in rats. The rats were treated at two dose levels continuously for life or for 8 or 16 weeks. The model was reasonably good in predicting the relationship between dosing period and latency of tumor formation when the data from the high-dose (20 mg/kg/day) group were used. It did not predict this relationship as well for the low-dose

group (6.7 mg/kg/day). The researchers concluded that the carcinogen's major effect was temporal displacement of the tumor incidence.

Temporal displacement may not be the only effect a carcinogen will have on tumor incidence. Littlefield et al. (117) found in their oncogenicity study of 2-AAF in mice, when examining the incidence of bladder squamous cell carcinoma, that discontinuing the 2-AAF dosing after only 9 months drastically reduced the number of tumors found compared to the incidence in mice continually fed 2-AAF for 24 or 36 months. However, they also found that early termination of dosing with 2-AAF did not substantially change the final incidence of hepatocarcinomas. These findings indicate that the carcinogenic mechanism of a single compound may differ depending on the tissue affected.

The differences in tumor induction found for 2-AAF in mice have also been discussed by Day and Brown (121). They examined the data from the study by Littlefield et al. (117) as well as animal studies and epidemiological studies on several other carcinogens. Using the multistage model for carcinogenesis as proposed by Armitage and Doll (122), Day and Brown calculated the effect of terminating exposure on tumor incidence according to the stage of the carcinogenic process affected by the compound. They showed, using this model, that discontinuing exposure to an early stage carcinogen would have much less effect on the future expected tumor incidence, while discontinuing exposure to a late stage carcinogen would drastically reduce the expected

rate of tumor formation. Using results from several published studies, they found that 2-AAF and benzo(a)pyrene acted like early-stage carcinogens for the induction of liver tumors in mice and skin tumors in mice, respectively. 2-AAF, DDT, and extract of stale tobacco smoke condensate acted like late-stage carcinogens for the induction of bladder tumors, liver tumors, and skin tumors, respectively. These results are of considerable importance for the estimation of risks to workers exposed to carcinogens, particularly when the effects of reducing or eliminating exposure are to be calculated. Day and Brown's calculations indicate that the effects of cessation of exposure to an late-stage carcinogen may be considerably greater than those from early-stage carcinogen. However, few experiments give sufficient data to determine whether the compound tested is an early-stage or late-stage carcinogen.

Crump and Howe (123) reported further refinements of the multistage model to permit utilization of the exact dosing schedule by which animals have been treated, rather than an average dose rate, to estimate the model's parameters. Using this approach, it is also possible to estimate risks for exposure patterns differing from that used in the experiment generating the incidence data. They presented formulas for determining the fractional equivalent of a lifetime's constant exposure represented by constant exposure for part of a lifetime commencing at any age and of any duration. Crump and Howe demonstrated that the magnitude of excess risk incurred by exposure to a

carcinogen depends upon the number of stages in the carcinogenic process, which stage is affected by the carcinogen, the age at which exposure began, and the duration of exposure. This method of adjustment is expected to be of great utility in performing risk assessments that more realistically reflect the patterns of human exposure. However, at present procedures for making this adjustment are available only for the multistage model.

6. Approaches to Defining Uncertainty of Interspecies Extrapolation

Recently, two approaches have been proposed for mathematical expression of the uncertainty associated with risk estimates derived through interspecies extrapolation.

Crouch and Wilson (124) have proposed an approach to quantitative risk assessment that would generate an explicit probability distribution for risk. Rather than simply multiplying an estimate of potency (or its upper limit) by an estimate of exposure to derive an expected (or "worst-case") risk, their method incorporates a probability distribution of species-to-species conversion factors, K_{ha}, and a probability distribution of possible exposures, d, with a probability distribution of animal potency, β_a, to give a probability distribution of human risk, β_h:

$$\beta_h = K_{ha}\ \beta_a{}^d.$$

Theoretically, this scheme is applicable to any functional expression of a dose-response relationship.

The dose-response model chosen and the set of experimental data selected determine the potency in a particular animal species and its probability distribution. Concerning the species sensitivity factor, Crouch and Wilson asserted that "by comparing tests performed with many chemicals in two species we may define a 'best value' for K_{12} for those particular species, together with the probability distribution for variations in K_{12}." As has been discussed, however, derivation of such a quantitative definition of differences in potency from species to species from existing data is not so easily accomplished, and, when one of the species of interest is humans, the data will be very limited.

To implement their proposed scheme, Crouch and Wilson specified several assumptions which could be varied if desired. In particular, for ease of computation, they assumed all the probability distributions in their model to be lognormal. They proposed that the measure of dose should be average lifetime dose in units of mg/kg/day and that the data should be fitted to the one-hit model. They then proposed selecting the set of data that yields the highest estimate of potency. They believe that reporting the upper 98th percentile on the derived risk distribution will encourage further experimentation to reduce the uncertainty.

The objective of deriving an explicit estimate of the probability distribution for the likelihood of a human risk of a given magnitude is commendable. However, Crouch and Wilson's

paper was preliminary and leaves for later development many of the difficulties involved in obtaining scientifically valid estimates of the parameters of this model. The appearance of mathematical precision associated with its results could create the impression that the uncertainties of biological reality have been accounted for to a far greater extent than they have been.

Lave (125) noted that the mathematical correctness of the statements of uncertainty generated by the model of Crouch and Wilson is conditional upon the accuracy of each of the component distributions selected. Therefore, "by not accounting explicitly for the assumptions in the conditional estimate, they bury important assumptions" and ignore a portion of the actual uncertainty. Hoel (126) pointed out that Crouch and Wilson have taken account of one aspect of error in the biological model by incrementing simple statistical variation with a factor for interspecies conversion, but they have omitted any variance component for a very important source of uncertainty, the choice of the mathematical model to express the dose-response relationship. Uncertainty due to selection of the dosage unit is also not explicitly addressed and is not adequately incorporated in the interspecies sensitivity factor (90, 127). Brown (127) commented that Crouch and Wilson's tentative selection of the one-hit model goes against their stated purpose of generating an estimate of risk against which benefits might be balanced, because the linear dose-response model used has

a pronounced conservative bias. The "best" estimate of risk for the stated purpose should ideally target the actual risk, while it would be left to the upper confidence limit to convey the degree to which that estimate was uncertain. Brown concluded, "The simplistic approach proposed by the authors does not begin to approach a reasonable solution to this problem" (127).

DuMouchel and Harris (119, 120) have proposed a Bayesian statistical method of simultaneously considering potency estimates on a number of related substances derived in parallel sets of testing systems to obtain an estimate of carcinogenic potency in humans for a substance for which epidemiology results are not available. This procedure could also be used to refine an existing estimate of potency and to quantify the uncertainty associated with such estimates due to experimental sampling error, unknown conversion factors among species, and the uncertain relevance of each type of experiment to the others.

Like Crouch and Wilson (124), DuMouchel and Harris (128, 129), took as their starting point a data set consisting of estimated potencies (i.e., slopes of dose-response curves). Thus, they did not directly confront the problem of selection of an appropriate dose-response model, but their test data consisted of potencies derived from a linear dose-response model. These potency estimates form a matrix (which may have empty cells) of substances versus test systems. For example, the data set they used to illustrate their method consisted of potency estimates for roofing tar emissions, coke oven emissions,

four types of diesel engine emissions, gasoline engine emissions, benzo(a)pyrene, and cigarette smoke; these were calculated from the results of epidemiology studies, skin tumor initiation experiments in mice, studies of enhancement of viral transformation in Syrian hamster embryo cells, and mutagenicity assays on mouse 5178Y lymphoma cells with and without metabolic activation. With this list of studies, they attempted to bring together information on potency from experimental systems far removed from the epidemiology and long-term rodent bioassays ordinarily employed in quantitative risk assessment.

They developed in detail a model for potency, that can briefly be described as:

$$\theta_{kl} = \mu + \alpha_k + \gamma_l + \delta_{kl}$$

where k indexes substance, l indexes test system, μ is the grand mean, α is the additive substance effect, and γ is the effect for the test system. The possible deviation or error δ, due to unique substance-test system interaction, is the key term in the model; if it is assumed that these observed errors are theoretically exchangeable among the substances or test systems, they can be used to estimate the degree to which the potency of a substance in humans might deviate from that predicted by the model. Prior probability distributions are assigned to the elements of the model to reflect the amount of preexisting information about their magnitude and variability; by using a procedure based on diffuse priors, DuMouchel and Harris needed to assume very little about the degree to which

these elements might vary. When the data are analyzed by this model, posterior distributions will be obtained that give best estimates of the factors in the model and their variances. In principle, such posterior distributions may later be used as new, more informed prior distributions when additional data have been obtained for analysis. A version of this technique has been applied in estimating the carcinogenic risks associated with diesel emissions (130).

The approach of DuMouchel and Harris was received enthusiastically by other workers in the field (131-133) for its comprehensiveness, its robustness, its ability to distinguish various sources of error, and its probable utility in identifying test systems that are in fact most reliable for cross-species extrapolation. Smith (133) and Kass (132) were most concerned that the validity of the exchangeability assumption be further investigated. Krewski (131) and Kass (132) pointed out that more attention should be directed toward the dose-response model used to generate the initial potencies, because use of a linear dose-response relationship is likely to lead to model misspecification error that would not be accounted for in the stated variances. In summary, Kass (132) said that this Bayesian method might "improve what advisors to policy makers do informally," but there would always be the "dangers of oversimplification, overstated precision, and neglect of beliefs other than the analyst's."

Although the concept of quantitatively combining results on various substances from different experimental systems may shock toxicologists and regulators, the approach of DuMouchel and Harris is more comprehensive and has a stronger statistical basis than that of Crouch and Wilson (124). This model has not, however, been developed to the point where it could be adopted into routine procedures for risk estimation. Both of these two new approaches require a number of assumptions to be made about dose-response relationships and about the form of the probability distributions. The application of either would require a degree of statistical detail in the information to be used in the model that is lacking for most carcinogens. Thus, although these approaches justify further development, they appear to be largely in the research stage. For practical purposes, one must necessarily rely on simpler techniques in most cases, while making explicit estimates of the range of variability inherent in risk estimates derived by interspecies extrapolation and using statistical techniques to do so where possible.

C. Selection of a Dose-Response Model for Low-Dose Extrapolation

1. Introduction

Some form of dose-response model is needed to extrapolate from observed data on tumor incidence at high doses to risks at low doses. A number of mathematical expressions of the

relationship between expected tumor incidence and amount of exposure have been proposed for this purpose. The determination of which of these, if any, is the most appropriate model to use is an important and controversial aspect of quantitative risk assessment methodology, because the models may predict risks at low exposure levels that vary by several orders of magnitude, while they fit the data in the experimental range of exposure almost equally well. For instance, at the hearings for the OSHA cancer policy, it was shown that the estimates of lifetime risk to humans from exposure to either vinyl chloride or saccharin varied a million-fold depending on which dose-response model was selected (71).

A mathematical model is expected to be consistent with the scientific information available about the phenomenon it is modeling. Since goodness of fit to the few data points generated by a bioassay does not generally provide a basis for selecting a model and the results obtained are strongly conditional on the model employed, the degree to which various dose-response models reflect our limited theoretical understanding of carcinogenic mechanisms is of great consequence in establishing their relative suitability.

2. Theories of Carcinogenesis and their Importance in the Selection of an Extrapolation Model

Ideally, the choice of models for high-to-low dose extrapolation should be determined by what is known or conjectured about the mechanism(s) of cancer induction. However, since the true mechanism of carcinogenesis is not known for any carcinogen,

uncertainty remains concerning the choice of the most appropriate model(s).

The tolerance distribution models such as the Mantel-Bryan (log-probit) or log-logistic models have as a basic assumption that the population has a distribution of tolerance levels to a carcinogenic substance. If the dose of a carcinogen exceeds an individual animal's tolerance level, it will develop cancer, but if the dose is below its tolerance level, cancer will not develop. Because of genetic, physiological, and/or environmental differences among individuals in a population, tolerance levels are also expected to differ. The dose-response function for the carcinogen may then be identified as an inverse measure of the frequency distribution of these tolerances within the population. Gaddum (134) and Bliss (135) independently proposed that such dose-response functions could be determined solely from consideration of the statistical characteristics of a study population.

Mantel and Bryan (136) proposed that a log-probit tolerance distribution of effects seen at high doses should be used for extrapolation to low doses, by assuming an arbitrarily shallow slope for the dose-response curve. A slope of one (in units of probits/log dose) was chosen for this extrapolation, on the grounds that it was shallower than those seen in experimental studies and thus would guard against the possibility that the true dose-response function at low doses might differ from that seen at high doses; it was also intended to allow for

the fact that inbred experimental animals are likely to display a more uniform response, and hence a steeper dose-response curve than the outbred human population to which the extrapolation is intended to apply. Because of this arbitrary choice of slope, the Mantel-Bryan model generally does not provide a good fit to experimental data; indeed it does not attempt to fit the experimental data. This is a major criticism of the model (137). While the log-probit model is based on statistical consideration of population responses, the log-logistic model was originally derived from chemical kinetic theory and its application as a dose-response model was proposed by Worcester and Wilson (138) and Berkson (139).

Neither of these tolerance distribution models is based on considerations of mechanisms of carcinogenesis except to the extent that they assume a mechanism consistent with a distribution of tolerances within the population. Several mathematical models have, however, been developed that incorporate mechanistic considerations. In particular, models were developed that were consistent with empirical data from epidemiological and experimental studies.

An important empirical observation is that in many human populations, the cancer death rate increases approximately with the fifth or sixth power of age (140, 141). To explain this phenomenon, several different mathematical models have been advanced. Fisher and Hollomon (140) proposed that a critical number of altered cells within a tissue is necessary for

a tumor to develop. This would explain the relation between cancer death rate and age as follows. If age is considered an indicator of total exposure, assuming roughly constant exposure level throughout life, then the chance of having one altered cell in a tissue would be proportional to total exposure, i.e., to age; the chance of having two altered cells in the same tissue would be proportional to the square of the exposure, i.e., to age^2, and so on. In general the rate of appearance of tumors would be proportional to (age)$^{n-1}$ where n is the critical number of cells. The theory of Fisher and Hollomon is expressed mathematically by the Weibull model. However, this theory is not consistent with the empirical evidence that cancers generally arise from a single cell (142).

A variation on the theory of Fisher and Hollomon (140) leads to the multi-hit model (143) which would explain the relation between age and cancer death rate while being consistent with the single cell origin of tumors. The multi-hit model is based on the idea that several "hits" on a cell are needed to produce cancer. A generalized multi-hit model based on the earlier work of Cornfield (143) was proposed by Rai and Van Ryzin (144). However, this model has been criticized for giving a poor fit to much experimental data (145).

The other model that attempts to explain the observed relationship between age and cancer death rate is the multistage model. Like the multi-hit model, the multistage model assumes that several events are needed to change a cell from normal

to cancerous. However, whereas the multi-hit model assumes that a carcinogen increases the likelihood of all of these unordered events ("hits"), the multistage theory is based on the observation that cancer is a progressive disease that develops in stages and assumes that carcinogens act by increasing the frequency of the progression from one stage to another. Nordling (141) proposed that progression through the stages was governed by mutational events and that a tumor appeared after a specific number of mutations had occurred in a cell with the mutations occurring according to a Poisson process. A similar theory was proposed by Stocks (146). The theories of Nordling and Stocks can also be expressed mathematically by a modified Weibull model. However, these theories imply that cancer incidence should be proportional not only to the sixth power of age but to approximately the sixth power of dose of carcinogen. This latter prediction (which also arises from the multi-hit theory) is inconsistent with experimental data.

To overcome this flaw, Armitage and Doll (122, 147) modified the theory of Nordling and Stocks by proposing that the transitional probabilities between the stages of tumor development were not all equal and that only some of the stages were dependent on the carcinogen while other stages had a spontaneous probability of transition independent of the presence of the carcinogen. This modification yields a model that is consistent both with the relationship between cancer death rate and the

sixth power of age and between cancer incidence and a lower power (first or second) of dose of carcinogen. Several variants on the Armitage and Doll model have since been proposed (see ref. 148 for a review), including one developed by Whittemore and Keller (148) that can account for the initiation and promotion phenomenon and differential effects of early- and late-stage carcinogens (121).

Another set of empirical data that has influenced model building comes from the work of Druckrey (116), who analyzed data from a number of animal studies involving high doses of carcinogenic nitrosamines. He observed that the daily dose rate was inversely proportional to a small power (typically 3, but ranging between 1 and 6) of the median latency period, the period between initial exposure to the carcinogen and detection of induced tumors in half of the experimental animals. A similar relationship had previously been observed by Bryan and Shimkin (113) with carcinogenic polycyclic aromatic hydrocarbons. Based on these observations, Jones and Grendon (149) proposed a model whereby the carcinogen interacts with cells rendering them potentially cancerous. Such cells then multiply to form a clone which would die out before giving rise to a tumor unless it coalesces with an adjacent clone. Since the number of potentially cancerous cells is considered to be proportional to the dose in a given volume of tissue, the mean distance between such cells would be proportional to the cube root of the dose, and the average time for coalescence of adja-

cent foci would be inversely proportional to the cube root of the dose, in keeping with Druckrey's empirical observation. Jones and Grendon (149) concluded that sufficiently low dose levels would be virtually without risk, because the time necessary for development of a tumor according to this model would exceed the lifespan of the individual. The theory of Jones and Grendon (149) is, however, inconsistent with the observed single-cell-origin of tumors. Further, Guess and Hoel (150) have pointed out that the apparent relationship between dose level and latency period can be easily explained not as a physical consequence of an increase in tumor growth time with reduction in dose, but as a simple mathematical consequence of the decrease in cancer incidence rate with decreasing dose. As the dose level is decreased, the probability increases that an animal will die of some other cause before a tumor develops. In order to assess the risk of developing a tumor before death from another cause, the distribution of the median latency period must be considered. Calculations based on the multistage model of carcinogenesis led Guess and Hoel (150) to deduce that even when the mean latency period was 10 times the normal lifespan, the probability of developing a tumor within a normal lifespan would be 7.1×10^{-4}, which cannot be considered a negligible risk. Another reason for questioning the applicability of Druckrey's formula to low dose rates is that Druckrey's empirical data were obtained with relatively high doses of potent carcinogens. Under these circumstances, a number of

tumors are initiated in each animal, but only the first to develop is recorded. The observed relationship is between dose and time-to-first-tumor, and reflects the multiplicity of tumors rather than a relationship between dose and time-to-median-tumor (71).

Several mathematical time-to-tumor models have been proposed assuming different mathematical expressions of the distribution of times-to-tumor. These include the lognormal distribution (151) and the Weibull distribution (152). Also Hartley and Sielken (153) proposed a general class of models of which the Weibull model is a special case. However, except in the case of superficial tumors, it is very difficult in practice to obtain accurate information on the time of occurrence of the tumor. In an animal bioassay, an internal tumor is diagnosed either when the animal dies or when it is sacrificed. Many types of tumor are not rapidly fatal. Hence, if a treatment reduces the lifespan of experimental animals in a bioassay, tumors may be detected earlier than in the control group that lives longer, giving the false impression of a reduction in time-to-tumor in the treated group. Because of this problem of confounding by competing causes of death, if time-to-tumor models for risk assessment are to be applied to the data generated by current standard bioassay designs, extreme care must be used to distinguish between incidental and fatal tumors.

A major influence on current theories of carcinogenesis is the work of the Millers (154, 155) who demonstrated that

most chemical carcinogens that are considered initiators either are electrophilic reactants or are metabolized to electrophilic reactants. Such substances can react with nucleophilic sites in the cell including DNA, RNA, and protein. Evidence that DNA is the most important target for carcinogenic action by electrophiles comes from several sources. (1) There is a strong correlation between mutagenic and carcinogenic activity (31). (2) Cancer is heritable at the cellular level, i.e., when a cancer cell divides its progeny maintain the same cancerous properties; also, cancers appear to arise from a single cell rather than from a mass of cells (142, 156) suggesting that a heritable genetic alteration is involved in the process of carcinogenesis. (3) In the case of cancers initiated by ultraviolet light, both carcinogenesis (157) and somatic cell mutagenesis (158, 159) are enhanced in the human disease xeroderma pigmentosum, which is characterized by incompetence of DNA repair systems at the cellular level (160). Xeroderma pigmentosum cells are also more mutable than normal cells by certain chemical carcinogens (158, 161). Also, ultraviolet light-induced carcinogenesis is inhibited by repair (photoreactivation) of specific DNA lesions (pyrimidine dimers) induced by the ultraviolet light (162). (4) For some organ-specific chemical carcinogens, the organ specificity correlates with tissue differences in repair activity for DNA lesions thought to be precursors of mutagenesis and carcinogenesis (163).

There is, thus, fairly convincing evidence for one major class of carcinogens that the chemical itself or an active metabolite reacts with and damages DNA and that this DNA damage ultimately leads to the development of cancer; hence, these are known as genotoxic carcinogens. For some other carcinogens, particularly metals, there is evidence to suggest that the critical DNA damage or alteration is caused not by direct reaction with the DNA but by interference with the fidelity of DNA replication (164).

Although these data imply that some form of DNA alteration is intimately involved in one likely mechanism of carcinogenesis, they do not provide information on the nature of the DNA lesion involved. The fact that many carcinogens can induce point mutations in bacteria does not necessarily mean that the induction of a point mutation is necessary and sufficient for carcinogenesis.

The evidence that for many chemicals interactions with DNA, possibly resulting in mutations, are involved in carcinogenesis provides support for a linear relationship between dose and tumor incidence. At least theoretically, a single molecule of a carcinogen should be able to cause a heritable change in DNA structure if the change occurred at a critical site and was not repaired. If such a change were sufficient for carcinogenesis, and assuming a linear relationship between dose and the number of DNA alterations produced, a linear

relationship between dose and tumor incidence with no threshold would be expected.

Cairns (165) has postulated that transpositions (mutations in the form of chromosomal rearrangements) are responsible for carcinogenesis by altering expression of large regions of the genome. Cairns (165) pointed out that, although some types of transpositions occurring in bacteria and yeast are not increased in frequency by mutagens, certain mutagens do induce large-scale genetic alterations such as translocations in mammals (166) and Drosophila (167), mitotic recombination in yeast (168) and fungi (169), and sister chromatid exchanges (170) and other forms of chromosomal rearrangement (171, 172) in mammalian cells in vitro and in vivo. Although such large-scale genetic alterations would not be detected in a bacterial point mutation assay like the Ames assay, most, if not all substances that cause such large-scale alterations also cause point mutations in bacteria. It is, however, possible that some chemicals may be found that cause transpositions but not point mutations. Such chemicals would not be detected in assays such as the Ames assay and other short-term tests would be needed to detect them.

With improvements in culturing tumor cells and refinements in banding chromosomes, it has been found that structural modifications in the genome that are clonal in nature and present throughout the disease process are associated with most human malignancies and that the exact modification is highly character-

istic of the tumor type (173). The possibility that these rearrangements may have a causal role in the development of cancer, rather than being a consequence of the transformed state, has been supported by studies of tumor cell lines from Burkitt's lymphoma patients and from rats and mice with an analogous condition, plasmacytoma (174). The c-myc gene (an oncogene) from human chromosome 8 is translocated into very specific areas of chromosome 14 coding for immunoglobulin genes that normally undergo transposition in the course of B-cell differentiation. Corresponding alterations in the expression of the c-myc gene product are observed.

The above results are representative of a multitude of dramatic insights into the molecular aspects of the mechanism(s) of carcinogenesis that have been gained in the past few years as a result of the rather unexpected coalescing of several lines of research (study of retroviruses, transfection assays of tumor DNA, genetic engineering, and cancer cytogenetics). Oncogenes, of which about twenty have been identified, are at the center of this productive research. These are nucleotide sequences found in the normal eukaryotic genome that are homologous to those in transforming retroviruses. These sequences are highly conserved in species as diverse as Drosophila and humans, which implies that their gene products play an essential role in the functioning of nonmalignant tissue. Carried by retroviruses or with a single specific base-pair substitution in the version normally in the human genome, oncogenes can

transform cells. It is possible that not all cancers involve oncogenes, but they provide an extremely useful system for studying oncogenesis at the molecular level. Studies of the molecular mechanisms of transformation by oncogenes have demonstrated that point mutation, chromosome rearrangement, gene amplification, and modification of the control of cell growth are all elements that, at least in some instances, play a role in carcinogenesis. It now appears that, although a single mechanistic pathway to malignancy is implausible, a fairly limited number of molecular mechanisms may be involved in the multistep production of more than 100 different tumor types by a variety of agents (175-179).

There is considerable evidence that some substances that do not directly cause DNA alterations can influence tumor yield. The most obvious example of this phenomenon is that of tumor promoters. In the classical mouse skin system, Mottram (180) demonstrated that after an initial application of a carcinogenic hydrocarbon to the skin of a mouse at a dose too low to induce a significant increase in tumors, multiple applications of croton oil to the same site would cause tumor formation, while croton oil alone was not carcinogenic. The major active ingredient in croton oil, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), is a powerful promoter, but is not mutagenic or carcinogenic by itself (181). This two-stage phenomenon on skin is species-specific; it apparently does not occur in rats, rabbits, or guinea pigs (182). However, there is evidence

that similar initiation-promotion events take place in the liver, esophagus, colon, bladder, breast, and stomach in the rat and the lung in the mouse (183). Such evidence that carcinogenesis is more than a single step process has led to several models embodying the multistep or multistage concept.

Two versions of a two-stage model for carcinogenesis involving an initial mutation followed by aberrant mitotic segregation resulting from promoter-induced increased frequency of sister chromatid exchanges (SCE) have been forwarded. These models differ in the consequence hypothesized to arise from the aberrant segregation of the genetic material.

Pall (184) proposed a mechanism of carcinogenesis for certain types of malignancy showing "homogeneously staining regions" and "double minutes" in their tumor cells. The model involves an initial mutation causing tandem duplication of a proto-oncogene, followed by gene amplification by unequal sister chromatid exchange until a sufficient number of copies of the gene and enough of the gene product are formed to transform the cell. Pall thus predicted that mutagens inducing large tandem duplications would increase the rate of the first step, and substances increasing the frequency of sister chromatid exchanges would increase the rate of the second step. It has recently been found that homogeneous staining regions and double minutes do contain multiple copies of oncogenes (185).

Radman and Kensella (186) have proposed a slightly different model, which is also a two-step process. The first step is

considered to be an event causing a recessive chromosomal change. The second step is considered to involve activation or induction of mitotic recombination leading to an increase in aberrant segregation and consequently to homozygous or hemizygous expression of the recessive change induced in the first step.

Both of these models rest heavily on the mechanism of sister chromatid exchange as the means by which aberrant segregation occurs. The evidence for this mechanism is largely the report by Kinsella and Radman (187) that the tumor promoter TPA induces sister chromatid exchanges. Unfortunately, the observation that TPA has this effect has been challenged by several authors (188-193).

Working with empirical data from the mouse skin initiation-promotion system, Burns et al. (194) found that increased incidence of tumors was modeled by a quadratic function in which the linear term was proportional to the administered dose of the promoter. This suggests that the risk posed by promoters may actually be of greater importance relative to initiators at low doses, contrary to the prevalent belief that the response curves for promoters might manifest thresholds.

In addition to the promotion phenomenon, there are several other situations in which substances that do not apparently interact directly with DNA increase tumor incidence. This has led to the suggestion that cancer is a disease characterized by impaired genetic control (195) or impaired cell differentiation (196); these may be caused by loss of responsiveness to growth-

control factors brought about by alteration in genetic expression (epigenetic mechanism) rather than alteration in genetic structure (genotoxic mechanism).

Evidence for epigenetic mechanisms of carcinogenesis also comes from the phenomenon of solid state carcinogenesis (197). Implantation of foreign bodies such as metal or plastic foils at various sites in rodents leads to development of sarcomas at the site of implantation. Research reviewed by Brand (197) has indicated that tumorigenesis is not related to the chemical properties of the material since the same substance did not cause tumors if powdered, shredded, or perforated. The physical properties of the implant are important since soft, rough, hydrophilic surfaces were less tumorigenic than hard, smooth, hydrophobic surfaces. The size and shape of the implant are also important; tumorigenicity was directly related to the surface area of the implant (197). Although the tumorigenicity of foreign body implants is not directly pertinent to human risk assessment, the possibly related phenomenon of carcinogenicity of inhaled fibers, such as asbestos, is. As with foreign body sarcomas, the tumorigenicity of fibrous particles is critically related to the dimensions of the material, and does not seem to be related to the chemical properties of the fibers, since chemically different fibers produce similar effects (198). However, not enough is known about the mechanism of asbestos-induced cancer to influence conclusively the choice of a method of risk extrapolation. Day and Brown (121) considered epidemio-

logical data on lung cancer in workers exposed for less than 2 years to asbestos and fitted the data to a multistage model to determine whether conclusions could be drawn concerning the stage or stages at which asbestos might act. The observed excess of lung cancer as a function of time after first exposure was consistent either with an early-stage effect or with a late-stage effect resulting from the asbestos that remains trapped in the lung after exposure stops. However, the observed dose-time relationships for the development of mesotheliomas in workers exposed to asbestos are more consistent with an early-stage effect (121, 199).

Reitz et al. (200) proposed an hypothesis for the mechanism of action of nongenotoxic carcinogens in which tissue damage leading to regenerative hyperplasia is responsible for the carcinogenicity of nongenotoxic chemicals. If it is assumed that there is a low but finite error rate in DNA replication, then increasing the amount of DNA replication, as would occur during hyperplasia, would increase the total number of replication errors. The effect of such an error might be analogous to that of a chemically induced DNA lesion produced by a genotoxic carcinogen. Alternatively or additionally, the increased rate of DNA replication would give less time for DNA repair systems to repair damage caused by the "natural" low level of genotoxic chemicals or physical agents (ultraviolet light, X-rays, gamma-rays) in the environment. It has been demonstrated that increasing the rate of DNA replication increases the suscep-

tibility of normal cells to mutagens in tissue culture (201). However, this hypothesis appears inconsistent with the low cancer incidence observed in young animals, which are experiencing most rapid tissue proliferation.

Such a model might also explain the mode of action of carcinogenic hormones such as estrogens (202-205), which stimulate DNA synthesis and cell division in their target organs. Promoters such as TPA also increase DNA replication and cell division (181); thus, such a mechanism might also explain the phenomenon of tumor promotion.

Based in large part on observations of the age distributions of cancers with familial and nonfamilial forms such as retinoblastoma, Moolgavkar (206) has proposed a two-stage initiation-promotion process of chemical carcinogenesis. He assumed that initiation involves a dose-dependent increase in the frequency of mutation. (In familial cancers, this initial mutation is inherited.) Promoting agents were assumed to induce modifications in the kinetics of cell growth, which might result in proliferative advantage for the mutated cell line or increased likelihood of a second mutation by indirect means.

Trosko and coworkers (207) have proposed that promoters have their effect by inhibiting cell-to-cell communication, probably by affecting the proteins of the gap junctions through which regulatory substances may pass from cell to cell. Peroxidation of the membrane by free radicals produced by the promoter might be the chemical mechanism of this effect (208). Metabolic

cooperation impaired in this fashion might result in an initiated cell having an advantage for growth that would permit its daughter cells to proliferate and achieve full carcinogenic potential, perhaps after additional genetic changes. These workers have developed an assay in Chinese hamster cells for inhibition of metabolic cooperation (209) that has given the anticipated positive results for several promoters, including TPA (209), benzoyl peroxide (208), and specific congeners of PCBs and PBBs (210).

Recently, data have been accumulating to support the hypothesis that free radicals may also play a role in the activity of promoters by inducing chromosome breaks, i.e., that promoters may indirectly have genotoxic effects (211). Application of TPA and other promoters produces an oxidative burst, the magnitude of which is correlated with the amount of DNA damage produced and the efficacy of the substances as promoters. This oxidative burst and chromosome breakage can be suppressed by inhibitors of promotion, such as retinoids. The DNA damage caused by these promoters is also prevented by compounds that block the formation of superoxide radicals. The breaks so induced by promoters might result in amplification of mutated genes or of oncogenes or in their increased expression.

The fact that many carcinogens must be metabolically activated to an ultimate carcinogen for activity and that these and others may be metabolically deactivated have led to the development of models that attempt to take such considerations

into account by using pharmacokinetic data to calculate from the crude dose (D_c) the active dose (D_a) of material available for reactions leading to carcinogenesis (109, 111, 202, 212-215). If only linear pharmacokinetics apply, D_a will be linearly related to D_c and pharmacokinetic considerations will not affect risk extrapolation. However, there is considerable evidence that for some chemicals D_a is not simply proportional to D_c at all dose levels (216). The topic of nonlinear pharmacokinetics is discussed elsewhere in this report. Where detailed pharmacokinetic data are available, such information may be used to transform the crude dose measure, D_c, to the actual dose of active material, D_a, and the transformed value of dose substituted in an appropriate mathematical extrapolation model. Such a procedure has been performed for the carcinogen vinyl chloride (109, 111). The use of pharmacokinetic data does not influence the choice of extrapolation model, only the measure of dosage used in calculations from the model. Gehring et al. (109) chose to use the log-probit procedure for extrapolation, but Anderson et al. (111) used both the log-probit and multistage models. The latter authors found that both models could be fitted to the pharmacokinetic data in the observed region of the dose-response curve, but gave widely divergent predictions in the low-dose region. Hence pharmacokinetic considerations do not help in the choice of a dose-response model, although they can improve the application of such models. Hoel et al. (213) have asserted, however, that after the admin-

istered dose is pharmacokinetically converted to the effective metabolite in the target tissue there is a linear relationshp between tumor response and the concentration of DNA adducts.

The foregoing review shows that there are a number of mechanisms by which substances may have carcinogenic effects. Genotoxic mechanisms include not only direct interaction with DNA, but also such indirect actions as inhibition of DNA repair. The mechanism of action of late stage carcinogens may involve the enhancement of differential growth of transformed cells. These considerations do not, however, provide guidance to the choice of extrapolation models except to emphasize that no single existing model may be appropriate for all carcinogens. The idea has recently been proposed that genotoxic carcinogens are qualitative dangers even at very low doses, while nongenotoxic carcinogens are effective only at higher doses, so that the two types of carcinogen should be assessed for risk and regulated differently (217, 218). As yet, however, risk assessment procedures are not sufficiently well developed for treating nongenotoxic substances differently, and, for any particular carcinogenic substance, it generally cannot be reliably demonstrated that it has no genotoxic activity (219). Only very specific information on the biological behavior of a given chemical would permit the choice of a dose-response extrapolation model with any degree of confidence that it reflected the mechanistic reality. The available information on mechanism(s) for carcinogenesis would suggest that the multistage model is the

most appropriate choice in the absence of data strongly indicating another model to be more suitable for a particular case.

3. Models Available for Low-Dose Extrapolation

a. Historical development

Over the past 30 years, several quantitative theories of carcinogenesis have been proposed to explain the time-of-onset or quantal response relationships with dose observed in the incidence of human cancer and in the results of experiments in animals. Several of these theories provide mathematical expressions that are useful in estimating carcinogenic effects which may result from low-dose exposures of humans.

The proposition that any toxic process may involve a threshold-type relationship, that is a dose level below which no response occurs, is generally not accepted for low-dose extrapolation for carcinogenic responses. The two main groups of nonthreshold models differ according to whether their primary concern is to account for the range of susceptibility in the exposed population (e.g., the log-probit and logistic models) or the mechanism of carcinogenesis (e.g., the multi-hit, multistage, and time-to-occurrence models). Additional models have been developed by combining features from these two main classes (e.g., the Weibull model). Most pharmacokinetic models describe the modification of dose within a living system, rather than the carcinogenic mechanism itself, and so are not directly used to extrapolate the incidence of toxic response to a given dose.

As is true for all models in science, models of carcinogenesis evolve as they are tested experimentally or are modified to account for new observations. However, because the mechanisms of carcinogenesis have not been completely determined experimentally, no single model can currently be claimed to be the most appropriate for all extrapolation.

Since quantile responses are bounded between 0 and 100% affected, relationships between dose and toxic response ordinarily can be described by a sigmoidal curve. Mathematical transformation of the data, such as expressing the response in normal equivalents (probits) versus the logarithm of the dose, usually gives a more nearly linear relation. The lognormal relationship is the basis for the carcinogenesis extrapolation method proposed by Mantel and Bryan (136). These authors considered the lognormal distribution to represent the distribution of tolerances (doses at which response occurs) of the individuals in the experimental population, so the low-dose relation between the probit of the response and the logarithm of dose should be linear. They suggested, for conservatism in extrapolation, that this line should be assumed to have a shallow slope, corresponding to a large variance for the distribution of tolerances. Thus, a shallow linear low-dose extrapolation, while perhaps not giving unbiased point estimates, would very likely represent an upper bound to the expected responses. A discussion of limitations to the method, including whether it is actually conservative (i.e., more likely to overestimate than underesti-

mate risk) was provided by Hoel et al. (220). The original method was modified by Mantel et al. (221) to account for spontaneous rates of tumor formation and to allow the combination of data from several experiments.

The second class of models, the "hitness" models, have their conceptual origin in the carcinogenesis mechanism proposed by Iverson and Arley (222). These authors postulated that cancer resulted from the single step transition of a normal cell to a precancerous stage as a result of a single "hit" by interaction with a carcinogen. Hence, the probability of such a transition would be a linear function of dose. This theory was supported by early experimental evidence that indicated that radiation-induced cancer displayed a linear dose-response relation. The initial extrapolation model assumed that a single "hit" was necessary; its use was discussed by Hoel et al. (220). A modification of this method is the multi-hit model, which requires more than one interaction to initiate the transition. The use of the multi-hit model, also called the gamma multi-hit model, in extrapolation was discussed by Rai and Van Ryzin (144, 223) and by the Food Safety Council (69, 70).

The multicell and multistage models are based on different concepts of carcinogenesis whose mathematical expressions are similar. The multicell mechanism, proposed by Fisher and Hollomon (140), postulates that a carcinogen induces similar transitions in many cells of a tissue and that a minimum number of these

cells must "coalesce" to form the nucleus of a tumor. Almost concurrently, Nordling (141) proposed a multistage process in which it is postulated that a carcinogen causes a single cell to undergo a number of transitions that eventually result in cancerous cells which can then proliferate with little or no inhibition. This mechanism is based on the observation of Nordling and others that the human mortality rates due to cancer increase as a power function of age, suggesting that the cancer seen in older people is the accumulated result of a multistage process begun earlier in life. Nordling's model assumes that the probabilities of the various transition stages are equal and proportional to the dose and length of exposure. Armitage and Doll (122, 147) proposed a modification in which the transition probabilities are not equal and in which it is assumed that some stages are dependent on the carcinogen dose and that some or all stages have spontaneous rates. Hence, transformation of the affected cell to a cancerous cell could proceed even after the initial carcinogen was no longer present. The use of the multistage model in extrapolation was discussed by Brown (224) and Guess and Crump (225, 226).

In their treatment of experimental data, all the preceding models assume that the administered doses are directly proportional to those that occur at the tissue level, i.e., the actual doses responsible for initiating the cancer. Research in pharmacology and metabolism demonstrates that it may be desirable to modify a model of carcinogenesis to address the types and

rates of metabolic transformations a carcinogen is likely to undergo. Gehring and Blau (108) and Gehring et al. (216) discussed the application of such pharmacokinetic concepts and the underlying assumptions that are made when the metabolism of a carcinogen is unknown, which, at present, is the most common situation. In the paper by Gehring et al. (109), pharmacokinetic analyses were applied to data resulting from the exposure of rats to vinyl chloride. Another model incorporating pharmacokinetics was advanced by Cornfield (227) in which the administered or proximal carcinogen is assumed to be either reversibly activated to an ultimate carcinogen that initiates carcinogenesis or detoxified via an irreversible process. The shortcomings of this approach were discussed by Brown et al. (228). Hoel et al. (213) have advocated the use of a pharmacokinetic model in order to estimate more precisely the tumor rate as a function of the concentration of DNA adducts in the target organ. Although this model is theoretically attractive, it requires detailed pharmacokinetic studies to establish a relationship between administered dose and concentration of DNA adducts. The practicality of this approach is doubtful, because the necessary pharmacokinetic studies have been performed on only a limited number of chemicals and the information on human metabolism will rarely be available.

The final class of models, the time-to-occurrence models, was developed to make use of all the information generated by carcinogenesis experiments and to satisfy the conceptual need

to account for the observed latency period between the administration of a carcinogen and the appearance of a diagnosable tumor. Druckrey (116) proposed that the probability distribution of tumor occurrence times would be a lognormal distribution. This model was applied to a number of experimental data sets by Albert and Altshuler (151). An extension of the Druckrey model was used by Jones and Grendon (149) to justify the existence of "practical" thresholds for carcinogenesis, i.e., human exposures that would pose no risk of cancer. The concept they advanced was that at sufficiently low doses the median time for tumor appearance would be greater than the average expected lifetimes of the individuals in the exposed population. Rebuttals to this concept were offered by Guess and Hoel (150), Gail (229), and Schneiderman et al. (230). Rather than the lognormal relation between dose and median time to tumor appearance, Pike (231) proposed the use of a Weibull distribution. Pike's method was elaborated by Peto et al. (152) and compared to the log-normal approach by Chand and Hoel (232). As a further improvement on the use of the Weibull distribution, Hartley and Sielken (153) developed the general product model which was discussed by Crump (233).

Many authors have pointed out that any extrapolation model needs to account for spontaneous or background levels of responses. One such modification is "Abbott's correction," which is an arithmetic transformation that assumes the independence of the doses responsible for the background responses and the

stimulus under investigation. A second modification which, assumes a positive additive relationship between the background doses and the stimulus, was proposed by Albert and Altshuler (151). A comparison between the application of these corrections and their outcomes was made by Hoel (234), who indicated that any additivity in dose would lead to substantially higher estimates of response at low doses than do the Abbott's correction models. Any assumption of additivity in dose leads almost directly to linearity at low doses.

Several authors have argued that carcinogenesis, in a manner similar to that assumed for other mechanisms of toxicity, should display a threshold dose below which no response is possible. This assumption would simplify low-dose extrapolation; only the experimental identification of a dose not producing an excess of cancer would be needed. Park and Snee (235) have proposed a "no-effect-level" model for use in establishing thresholds for effects of nongenotoxic carcinogens, but the method by which the "estimated no-observed-effect level" is obtained was not specified. Other researchers (236, 237), have pointed out that extrapolation of a threshold from animal experimentation must account for a wide variation in response in the exposed human population due to genetic and environmental factors. Mantel and Bryan (136) and Brown (236) have shown that such population variation would be expected to cause the dose-response curve to be more convex at low doses and thus more similar to the linear extrapolation models which deny

the existence of a threshold. No mathematical formulation of a threshold dose-response relation has been advanced, except that Hoel et al. (213) showed that under certain restricted conditions on pharmacokinetic parameters, their pharmacokinetic model could yield quasi-threshold type dose-response curves.

b. Description of individual models

(i) The Mantel-Bryan probit extrapolation model

The Mantel-Bryan extrapolation method was proposed (146) as a procedure for estimating the lower confidence limit for a "virtually safe" level of exposure to a carcinogen, "virtually safe" being defined as a predetermined small increase in lifetime risk. The dose-response function used in this method is the probit (lognormal) distribution of tolerances of the exposed or studied population. This relation has as its graphical description a sigmoidal curve and has wide empirical support in toxicology. If all members of the population are exposed to a given dose, d, then the proportion of responders or probability of response, P(d), will be:

$$P(d) = c+(1-c)\int_{-\infty}^{a+b\ \log_{10}d} (2\pi)^{-1/2}\exp(-x^2/2)\,dx$$

The model has an intercept parameter a, a slope parameter b, and a background parameter c representing the probability of a response in unexposed animals. The slope can be interpreted as the reciprocal of the tolerance distribution standard deviation and, hence, small variability in the distribution will produce a large slope and large variability a shallow slope.

In their paper, Mantel and Bryan proposed that the slope of the probit curve should be set at 1. The rationale for doing so was that their method was intended to avoid the overestimation of safe levels that may result from extrapolation from a curve with a large slope. Their experience with other biological dose-response curves from experiments with homogeneous laboratory animals indicated that a slope of 1 was much less than those usually observed and therefore would provide the desired conservatism. The overall original Mantel-Bryan procedure then was to determine from the experimental data the highest dose which yielded no response, calculate the maximum risk associated with that dose at the 99% confidence level, and then use the probit function to extrapolate to a dose that would yield a risk of 10^{-8}. This procedure was improved by Mantel et al. (221) to account for spontaneous responses, i.e., those occurring in the absence of the carcinogen, and to use the data at all dose levels in a more rigorous way. These goals were accomplished by first using all the data, including the control data which provide an estimate of the spontaneous response, determining a maximum likelihood estimate for a, the intercept parameter, and then finding the upper 99% confidence limit for a (called a*) using a chi-square distribution with a single degree of freedom. The safe dose is then the 99% lower confidence limit corresponding to the dose giving a 10^{-8} response using a* in the probit model.

As pointed out by Mantel and Schneiderman (86), the Mantel-Bryan approach has several advantages in making extrapolations for regulatory purposes. First, rather than regarding a carcinogen as unsafe at any dose, it asserts that there should be a dose small enough that the risk is acceptable to society. Second, through use of confidence limits, the procedure encourages larger, more thorough experiments which reduce the variability in the data and, on the average, lead to determination of a higher safe dose. Lastly, it is easy to use and applies a single rule to all circumstances.

However, as indicated by several authors, the procedure has several severe shortcomings. Most importantly, the Mantel-Bryan model is arbitrary and has no basis in the biology of carcinogenesis. As pointed out by Crump (238), the model typically does not fit experimental data well. Hartley and Sielken (153) pointed out an important limitation of the probit function, that it becomes very shallow as the dose approaches zero. Hence, if the true dose-response curve is an analytic function, which is likely, then the probit curve will eventually underestimate the true risk regardless of the experimentally-derived parameters or the chosen probit slope. Hence, extrapolation based on this curve will be likely to yield a larger safe dose than an extrapolation curve more closely fitted to the experimental data.

(ii) Linear extrapolation

Several methods for the extrapolation of carcinogenicity data have been developed that depend on the assumption of a linear relation between dose and response. In 1970, Gross et al. (239) published a proposal for estimating safe levels of food additives shown in tests to cause irreversible effects such as cancer. Their basic assumption was that the unknown, low-dose portion of the response curve is most likely concave and that a procedure based on a linear relation in this region is certain not to underestimate the risk. Therefore, they suggested fitting a least squares regression line to the observed data and then calculating an upper limit on its slope, b_u. This slope is then taken to be the slope of the dose-response curve at the origin. The safe level of exposure, then, is found by dividing the selected limit of response by b_u. Further, the authors advised sequentially eliminating the highest dose group if use of only the lower doses yields a lower value of b_u.

As pointed out by Hoel et al. (220), the Gross procedure is essentially a method for arriving at the parameter q of the one-hit model, which has the following expression for determining the probability of a response at dose d:

$$P(d) = 1 - \exp(-qd).$$

This expression becomes

$$P(d) = qd$$

at very small values of qd, i.e., low doses. The major criticism of this linear approach is that, because it assumes a linear response in the observed range (in essence manipulating the observed response to obtain a shallow-sloped linear relation), it will always yield very low safe doses at low levels of acceptable risk. As indicated by Hoel et al. (220), application of linear extrapolation to a single dose assay in which no carcinogenic response was observed would produce exceedingly small allowable exposure limits. Hence, exposure to many substances would be restricted even if no positive indication of carcinogenicity was obtained.

Unlike the linear extrapolation discussed above, some linear methods are used to arrive at estimates of low-dose responses without regard to the shape of the possible curves fitted to the observed data. The basic premise of a procedure proposed by Gaylor and Kodell (240) is that the most likely form of the low-dose portion is concave-up, and hence, a line connecting the lowest point on the observed curve and the zero-dose response will lie above the true curve. Thus, the process is intended to identify the upper limits of the potential risk, i.e., to overestimate the risk. The authors suggested using any model to fit a curve to the data and obtain an upper confidence line for the observed region. Then linear interpolation is applied between the estimated upper limit on response at the lowest experimental dose (adjusted for the background tumor rate) and zero to find "safe" doses corresponding to a specified

risk. In their examples, the authors used the Armitage-Doll multistage model to estimate the lower limits on dose for the given risks. In 11 out of 14 cases, contrary to what might have been expected, their estimates of "safe" doses were less conservative (higher) than those obtained with the multistage model; this was attributed to the wide asymptotic confidence bands of the multistage model.

The advantages of this linear extrapolation procedure are its ease of application and that it can be used even when only one dose level was tested. The main criticism of the procedure is that the selection of the starting point is arbitrary and totally dependent on the chosen curve-fitting method. For example, with the Armitage-Doll model, the width of the confidence limits are dependent on the choice of the parameter representing the number of stages (241).

(iii) Multistage and multi-hit extrapolation models

The theory that carcinogenesis is a multistage process provides the basis for multistage extrapolation models. Initially proposed by Nordling (141), this theory postulates that a single cell must undergo a certain number of changes prior to becoming malignant and capable of growing to a tumor. A mathematical description of this process that relates incidence to time was given by Peto (242). If n is the number of stages in the process and R is the event rate for each stage (or the probability that a cell will undergo a change to the next stage), then the incidence of cancer in a population at time t is

$$I(t) = R_1 R_2 \ldots R_n t^{n-1}$$

This expression assumes that the event rates are constant throughout the life of the organism. A carcinogen, then, can act to increase any of the event rates.

A more general expression accounts for the more plausible biological situation in which some cells at a given stage have a selective advantage over their normal neighbors and increase in number more rapidly. Peto (242) proposed that this increase is proportional to the square of time since the origin of that clone of cells and, hence,

$$I(t) = R_1 R_2 \ldots R_n C(t)^{n+1}$$

An alternative expression that relates the incidence to the dose of the carcinogen by assuming that the individual event rates are all directly proportional to the dose (d) was developed by Armitage and Doll (122). After continuous exposure, the cumulative incidence is approximately

$$P(d) = 1-\exp \sum(-\beta d^n).$$

This form can be modified to account for spontaneous rates of change by inclusion of parameters independent of dose in the exponent:

$$P(d) = 1-\exp \left[-\prod_{i=1}^{n} (\alpha_i + \beta_i d) \right],$$

or, more generally,

$$P(d) = 1-\exp\left(\sum_{i=0}^{n} q_i d^i\right).$$

When the model is fitted to data, the following constraints are observed: $q_i \geq 0$ and n is one less than the number of dose groups (243).

In extrapolation, the most important feature of this expression of the multistage model is that the response curve is essentially linear at low doses when q_1 is greater than 0, i.e., the slope of log [P(d)-P(0)] versus log (d) is 1. If q_1 is equal to 0, as is the case for positive curvatures, the log-log slope at low doses will be 2 or more; however, upper confidence limits on risk will be linear in dose in all cases (244). Because of the number of exponential terms possible, the multistage model can fit many sets of experimental data, both for animals (224) and humans (242). A special case of the multistage model is the single-hit form which is discussed below.

There are three general criticisms of the multistage model and its utility for low-dose extrapolation. The first, and this can be said of all existing models, is that its central concept, the "stage", is biologically undefined. Peto (242) noted that a stage has to be long and transitions infrequent. For human cancer, he believed that a stage must be between 1 and 10 years in length. As yet, there is no suggested biological mechanism for carcinogenic events at the cellular level that would occur on this long time scale. The result, as argued

by Carlborg (245), is that the model is mathematically undefined, i.e., the number of stages is arbitrary and depends only on how many stages one chooses to use in fitting the model to a given data set. As an example, he presented 12 of infinitely many solutions of the model for a given data set, all of which gave different low-dose risk estimates. In fact, the number and degree of exponential terms in the multistage model as currently used (226, 246) is required to be smaller than the number of data points, so none of the "solutions" Carlborg gave for his example would be considered possibilities; well-defined maximum likelihood procedures determine which power terms will be present in the best-fitting model and their coefficients (246). The uncertainty in the form of the polynomial exponent in the observed region produces greater uncertainty in extrapolation. A second criticism is that the estimated response at low doses is insensitive to the shape of the curve in the observed dose-response region, i.e., widely different observed curves will yield similar extrapolated risks (245). Finally, although the multistage model can be applied to many sets of data successfully, it does not fit response curves that have an initial steep portion and then flatten out (245). However, some of these curves can be fitted to the multistage model if pharmacokinetic information is utilized (111).

The development of the multistage models has been paralleled by that of the multi-hit models, which are based on several assumptions: (1) an organism has critical sites which, when

altered, will result in cancer induction; (2) a site is altered if it is hit by k toxic particles; and (3) the probability of a hit is proportional to dose (222, 223). A special case of the multi-hit model is the single-hit model which has the form:

$$P(d) = 1-\exp(-qd).$$

This is also recognizable as a special case of the multistage model with only one stage. However, this expression with a single adjustable parameter, q, will often not fit experimental data, and will always produce a linear low-dose extrapolation. The multi-hit expression accounts for the probability of the occurrence of k hits by using a Poisson distribution. It has the form:

$$P(d) = c+(1-c)\int_{o}^{qd} x\frac{(k-1)}{(k-1)!}\exp(-x)\,dx$$

where c is the independent background incidence. This model can be generalized to a tolerance distribution conforming to the gamma distribution by assuming that k is not necessarily an integer. This model has been applied to a number of data sets (69, 70, 223) and has the advantage of fitting a wider variety of curves than the multistage model. As discussed elsewhere, the application of the gamma multi-hit model involves Abbott's formula for incorporation of background response rates.

Several problems that arise from the use of the gamma multi-hit model were pointed out by Haseman et al. (145). After reviewing the Food Safety Council report (69), these authors noted that the procedure may "manufacture" a background rate, where the data do not indicate one, which is then used to dismiss a possibly significant increase in tumors at low doses. In addition, by allowing the number of hits (k) to be less than one, the model produces unrealistically high estimates of "virtually safe dose" (VSD). Haseman et al. used vinyl chloride as an example, and showed that the VSD estimates with and without consideration of the high dose responses differed by a factor of one million. Further, the authors pointed out that a reliable procedure for calculating low-dose confidence limits for the gamma multi-hit model has not yet been characterized. Crump et al. (89) further remarked that, if the k hits were modeled to occur in a single cell rather than a tissue and if the background were made additive rather than independent, the multi-hit model would become identical with the multistage model, thus implying that it has less theoretical justification than the multistage model.

(iv) Time-to-tumor extrapolation models

Time-to-tumor extrapolation models are derived from attempts to model the relationship between dose and the time needed for the response to occur. The first to apply this line of thinking to carcinogenicity data was Druckrey (116) who reported

an empirical relationship between dose (d) and median time of tumor appearance (t) to be:

$$dt^n = \text{constant}$$

where n is usually between 1 and 6. Druckrey assumed that the probability distribution of the response times was a log-normal distribution:

$$f(t) = (2\pi\sigma^2 t)^{-1/2} \exp \left[-1/2 \left(\frac{\log(t)-\log(\mu)}{\sigma}\right)^2\right]$$

where μ is the median time of tumor occurrence and σ is the standard deviation of the log response times. This expression is similar in form to the lognormal population tolerance models discussed earlier. Albert and Altshuler (151) applied this model to several data sets from both animal experiments and epidemiology and found good agreement.

Extension of the model for low-dose extrapolation purposes was proposed by Jones and Grendon (149). After examining data of their own and of Druckrey, they generalized that the median time-to-tumor appearance is proportional to the 1/3 power of the dose and proposed that this relation reflects the average time needed for two affected cells to coalesce to form a cancerous clone. Consequently, if this relation holds for most cancers, they concluded that "low dosage exposure at some levels is virtually without risk because the expected lifespan of those exposed is exceeded by the time necessary for low concentrations of altered cells to develop into cancers."

Guess and Hoel (150) examined this proposal in the context of making assessments of risk at low doses. Examination of

the qualitative relationships between the variables led them to the conclusion that "the first time-to-tumor and the mean time-to-tumor necessarily increase with decrease dose as a consequence of the decrease in incidence with decreasing dose," regardless of the time distribution function used. Further, their calculations show that, even if the median time-to-tumor is much greater than the animal's lifespan, this does not mean the risk is negligible, i.e., at a dose providing a long time-to-tumor, a substantial risk would still exist. In addition, Schneiderman et al. (230) pointed out that as the time distribution broadens the meaningfulness of the time-to-tumor model for extrapolation decreases. Thus, for the genetically heterogeneous human population, the distribution is most likely broad and the time-to-tumor model less applicable than other models. A further constraint on the use of time-to-tumor models is the practical difficulty of gathering this type of data from the standard bioassay design, as discussed earlier.

Another distribution function that applies to time-to-tumor data is the Weibull distribution. Pike (231) obtained the Weibull distribution as a mathematical model of carcinogenicity by assuming that cancer occurs when the first of n independent cells in a tissue becomes cancerous. He derived the following probability density function, g(t), for time-to-tumor, t:

$$g_n(t) = n\, f(t)\, [1-F(t)]^{n-1},$$

where f(t) and F(t) are the probability density function and cumulative distribution function, respectively, of time to cancerous transformation in a single cell. According to the asymptotic theory of extreme values, three distributions satisfy this equation, among which Pike selected the Weibull as being most biologically feasible, since human cancer mortality rates rise as a power of age. The Weibull cumulative distribution function for the time-to-tumor, t, is:

$$G(t) = 1 - \exp[b(d)\ (t-w)^k],$$

where b, the inverse of the Weibull scale parameter, is a function of dose, d; k, the shape parameter, is independent of dose; and w can be considered a latency period.

The Weibull model was originally developed in the context of time-to-failure for components of engineering systems. It is a Type III extreme-value distribution, i.e., the distribution of the minimum among identically distributed random variables that have a lower bound on the value they may assume. It models the time to the first "failure" in a multicomponent system (247), here the first cell to be transformed. Its application to carcinogenesis has been primarily empirical, since it does not incorporate theoretical considerations of the underlying biological mechanisms of carcinogenesis. However, it corresponds mathematically to a multistage model in which the penultimate transition is followed by a constant latent period rather than a stochastically occurring final event (152, 231). The Weibull distribution can also be derived from the multi-

hit model (140, 143, 144). The Weibull model is useful for application to sets of data in which there is extensive information on cohorts of different ages, and it has been applied successfully to the results of epidemiological studies (248, 249). It is equally applicable to animal data, although animal bioassays are usually designed in such a way as to minimize the range in ages at death. Peto et al. (152) compared the fit of the Weibull model to various sets of data and found it to fit better than the lognormal distribution.

Hartley and Sielken (153) derived a general product model which has the expression:

$$F(t) = 1 - \exp\left[-\left(\sum_{j=0}^{k} q_j d^j\right) h(t)\right].$$

The Weibull model is a special case in which $h(t) = kt^{k-1}$. Although it is of theoretical interest, use of the general product model would require prior selection, rather than data-dependent estimation, of three of its parameters; therefore, study of the effects of these essentially arbitrary choices on estimated risks needs to be made (89).

A critical problem in the utilization of time-to-tumor models is the need to account for the possibility that tumors might have formed later in "censored" animals, that is, animals dying or sacrificed, but found not to have a tumor. Daffer et al. (250) proposed a method for accomplishing this by using a nonparametric distribution function.

As mentioned previously, a constraint on the use of time-to-tumor models is the difficulty of determining the actual response times in an experiment. Internal tumors are difficult to observe in live animals and their presence is usually detected only at necropsy. Also, the application of these models often requires making a distinction between whether a tumor was the cause of an animal's death or merely found coincidentally at necropsy when death was due to other causes; pathologists are frequently reluctant to draw such conclusions. Further, competing causes of death, such as toxicity of the test compound, may decrease the observed time-to-tumor of nonlethal cancers by allowing earlier necropsy of animals in higher dose groups.

4. Major Issues in Selection of Dose-Response Models

a. Thresholds

It has frequently been asserted that for any toxic response there exists a threshold, i.e., a dose level below which it is biologically impossible for a response to occur. As the mechanistic nature of carcinogenesis and mutagenesis have become better understood, it has seemed less reasonable to assume that this assumption would be valid for these effects.

Several properties of the carcinogenic process make it seem likely that an arbitrarily small amount of a carcinogen, even a single molecule, could effect the transition from one stage to the next and that a greater amount of the substance merely increases the probability that such an event will occur. Cancer has a single cell origin, the damage being heritable

and irreversible in somatic cell lines, although it may not be manifested until a long latency period has elapsed. This differs from mechanisms of toxicity that work by exceeding the capacity of a biological system, e.g., the immune or detoxification resources, thereby producing a response at dose levels above that capacity and no response below that capacity. A true threshold would exist only if the defenses were perfect up to that capacity.

The phenomenon of the latency period has been offered as the basis of a "practical threshold," since the observations of Druckrey (116) indicate that in experimental animals the median time-to-tumor appearance is inversely related to dose. The implication is that the time-to-tumor would be so long for very small doses of a carcinogen that it would greatly exceed an individual's lifespan, making the exposure effectively safe. It has been shown (150, 230) however, that for a non-trivial portion of the population, the individual times-to-tumor would fall within the average lifespan even when the median greatly exceeded it.

Another biological argument for the existence of thresholds for carcinogens is that some known carcinogens are required in small amounts for normal functioning and must therefore be safe at those levels (251). There is no obvious reason, however, why the beneficial and carcinogenic actions of such substances should be mutually exclusive (230). It has been shown, for instance, that the length of time a woman is exposed

to her own naturally produced estrogen is related to her risk of breast cancer (252).

Thresholds for any type of effect are not experimentally demonstrable. With sufficiently large groups of animals, arbitrarily small increases in incidence could theoretically be shown to be statistically significant. The absence of an effect, however, cannot be proven. Only an upper probability limit can be put on the size of an effect when statistical significance is not found, and the amount by which this limit exceeds zero is determined by the power or size of an experiment. Therefore, if a threshold did exist for a set of identical individuals, as approximated by highly inbred laboratory animals, it would be virtually impossible to locate the dose at which it occurred.

Furthermore, even if each individual had a threshold defined by his physiological constitution, the population would be represented by a distribution of thresholds with no absolute lower bound or population threshold (236, 237). In outbred populations such as humans, genetic heterogeneity is so great that the tolerance of the most sensitive individual could not possibly be estimated, and the dose-response curve would not be distinguishable from nonthreshold concave-up models (136). In fact, it has been suggested (137) that, if the thresholds exist, they may characterize the cell, making these arguments about population thresholds applicable to the individual organism.

Another factor that makes the existence of thresholds seem extremely implausible for carcinogens is the substantial

"background" incidence of cancer in humans. Unless each carcinogenic substance operated by a unique mechanism, an additional small exposure to a substance would supplement an individual's exposure to other similar carcinogens. From the high incidence of cancer of unexplained etiology, it is clear that the human exposure is well in excess of any possible population threshold. Viewed in this manner, no additional exposure, no matter how small, could be completely without risk (234).

For these reasons, it is not scientifically justified to entertain the idea that the true dose-response curves of all carcinogens have thresholds or to consider extrapolation models that postulate them.

b. Additive versus independent dose

In animal bioassays used as the source of incidence data for low-dose extrapolation, a non-zero incidence of the tumor in question will frequently be observed in the controls. In the human population also there is a sizeable incidence of cancers that are not clearly attributable to any specific agent or cause. In estimating the incremental risk due to a particular substance, it is necessary to take account of this background incidence in some fashion. Beyond dealing with existing background, this issue extends to the question of how particular agents interact and whether cumulative exposure to "virtually safe doses" of several substances might in fact be unsafe.

Two general approaches have been taken to this problem. The first assumes that the response, $P^*(D)$, elicited by an

amount D of a substance is totally independent of the background incidence, P_o. The observed incidence at dose D, P(D), is then expressed by Abbott's correction (253) as:

$$P(D) = P_o + (1-P_o)\ P^*(D).$$

The second approach is to assume that the observed response is the result of the administered dose, D, augmenting an ongoing process that has already been stimulated by a hypothesized amount of "background dosage", D_o:

$$P(D) = P^*(D + D_o).$$

This additive hypothesis for the action of individual carcinogenic agents was proposed by Albert and Altshuler (151). Peto (254) suggested a biological rationale for additivity of dose by noting that it is very implausible that biochemical endpoints can only be reached in a single way or that each carcinogen operates by a unique mechanism.

Discrimination between independence and additivity is often difficult on the basis of observed dose-response data, but incorporation of this hypothesis into an extrapolation model can have a marked influence on the resulting estimates of risks at low doses. Hoel (234) illustrated this point using a lognormal dose-response model.

In the one-hit model and the multistage model either assumption leads to low-dose linearity. Crump et al. (137) have demonstrated that the assumption of additivity will result in low-dose linearity in any model. When Hoel (234) investigated the consequences of assuming that a portion of background incidence

was due to additivity and the remainder due to independence, he found that assuming any of background to be additive resulted in low-dose linearity. This would imply that the mechanism of the background must be totally independent of the mechanism of the dosing agent, if the dose-response curve is not to be linear in the low dose range.

Crump et al. (137) asserted that, if a carcinogenic agent affects the cellular mechanism that produces background incidence, additivity should be assumed. Mantel (255) took issue with this recommendation, saying it was not scientifically established and that there are biological processes such as cellular repair that could prevent the occurrence of low-dose linearity. The Food Safety Council (70) also objected to the universal assumption of additivity, and consequently low-dose linearity, by making note of such biological phenomena as separate inactivation pathways for different chemicals. They concluded:

> From the regulator's point of view it would appear as if additive joint effects are not so common as to be automatically assumed, but that when their existence, and hence low dose linearity, are suspected, this would influence the choice of mathematical models. (70)

It is currently unclear whether additivity or independence of dose is the general rule, and the question is not readily testable for a given substance. It seems likely that some intermediate situation represents the state of nature. Given the conservativism of dose-response curves with low-dose linearity and the fact that if any of background is additive a model

becomes linear at low doses, it would seem prudent to follow the reverse of the Food Safety Council's recommendation and to assume additivity unless independence can be convincingly demonstrated for a given substance.

c. Low-dose linearity

The issue of low-dose linearity involves many of the points discussed previously in connection with the questions of thresholds and additivity-versus-independence of dose. A dose-response curve has the property of low-dose linearity if a line tangent to it has a non-zero slope at zero dose.

The existence of a background incidence of tumors has been propounded as a theoretical reason why dose-response curves should be expected to be linear at low doses (137, 254). Watson (256) also showed that low-dose linearity would be the case in the presence of background whether heritable changes were reversible or irreversible or whether a sequential or nonsequential model was assumed. Cornfield and Mantel (257) contended, however, that a biologist would be frequently inclined to hypothesize that the slope of the dose-response is strictly zero at zero dose, which is equivalent to assuming that a threshold exists.

A dose-response curve that has a non-zero slope at a dose of zero will lie above any other dose-response curve not having this property and so being concave-up near zero. A low-dose linear curve will, therefore, give higher risks in the low-

dose range and hence can be considered a conservative upper bound for risk extrapolation.

Van Ryzin (258) has summarized the low-dose behavior of the commonly used extrapolation models. In the low-dose range, the one-hit model is linear under all circumstances and the multistage model is linear if it contains a linear term. The multi-hit, logistic, and Weibull models may be concave-down, linear, or concave-up, depending on the data set to which they are fitted. The log-probit model always approaches zero faster than any power of dose.

The Food Safety Council (70) disputed the frequently held opinion that an extrapolation model should always be linear in the low-dose range.

> ...low-dose linearity would most likely be limited to classical electrophilic carcinogens, i.e., those acting through a mutation-like event. Such responses would be difficult to imagine with substances which are carcinogenic as the result of prolonged tissue damage. Application of this philosophy in all cases is considered to be unwarranted, and overly conservative, since in some instances low-dose linearity can be excluded on biological or metabolic grounds. (70)

Although the Food Safety Council argued in this passage that low-dose linearity would be expected only for carcinogens that interact directly with DNA, in fact many of the arguments for low-dose linearity are equally applicable to other proposed mechanisms of carcinogenesis. In particular, dose additivity would lead to low-dose linearity for carcinogens acting by any mechanism for which there is a natural background frequency (e.g., enzyme induction, inhibition of DNA repair, sister chroma-

tid exchange, hormonal action, cell proliferation, and even gross tissue damage). This concept is expressed mathematically in the multistage model, which predicts low-dose linearity for carcinogens acting at any stage. (However, this result derives directly from the assumption of linearity built into the model.) Empirically, there is evidence for linearity for certain late-stage carcinogens even at relatively high doses (121). The key issue in the choice of models is whether dose additivity can be assumed to be absent (i.e., whether a carcinogen can be assumed to act by a mechanism completely independent of background events). In this respect, it would seem reasonable to adopt the conservative approach of assuming low-dose linearity unless in a particular situation it can be shown that it is not applicable.

d. Competing risks

Competing causes of death may obscure the true nature of a substance's carcinogenicity, or lack thereof, when bioassays or epidemiology studies are analyzed. In general there are other, not necessarily substance-related, causes of death that may remove a subject from observation before sufficient time has elapsed for the other alternative, a tumor, to be manifested.

If a substance under study has toxic effects in addition to carcinogenicity, the treated animals may be killed before tumors have become detectable. Therefore, analysis of the data on the basis of simple presence or absence of a tumor might lead to the conclusion that the substance had no effect

or even inhibited tumor formation. An alternative time-to-tumor analysis might reveal the true situation more clearly (233).

On the other hand, if a time-to-tumor model were used to analyze data on a noncarcinogenic, but otherwise toxic, substance with the endpoint defined as presence of a tumor at time of death, an incorrect conclusion that the substance was carcinogenic might be drawn. This could happen if the other toxic effect killed the treated animals sooner, whereby a crop of background or spontaneous tumors might be found earlier than in the controls (233).

For this reason, it may be useful to consider time-to-occurrence data in addition to the dichotomous variable of tumor presence or absence (151, 229), but the endpoint must be clearly defined (observed tumor on a living animal, lethal tumor, tumor found at time of death, etc.) and the results must be interpreted carefully (259).

e. Application of models to various data sets

To gain insight into the variation in low-dose risk estimates produced by different models, several authors have applied low-dose extrapolation models to available data from carcinogenicity studies or to hypothetical data sets. This section summarizes their findings and indicates some general conclusions that may be drawn about the comparative behavior of different models.

Chand and Hoel (232) compared the lognormal and Weibull models modified for competing causes of death by applying them to an hypothetical data set. The estimates of risk given by the two models diverged considerably at low-dose levels, with the lognormal model giving risks 8 orders of magnitude lower than the Weibull at a dose level 7 orders of magnitude below the range where the two curves originated.

The Food Safety Council compared the one-hit model, the Weibull model, the gamma multi-hit model, and the Armitage-Doll multistage model (69, 70). These models were applied to tumor incidence data on nine carcinogenic compounds. (Non-carcinogenic endpoints were considered for five other compounds.) In eight cases, the Virtually Safe Doses (VSDs) for risks of 10^{-4} and 10^{-6} decreased in a regular pattern: multi-hit > Weibull > multistage > one-hit. Vinyl chloride was the only exception and the VSDs in this case fell in the reverse order. The lower confidence limits on the VSDs were also compared. Depending on the data set, the Armitage-Doll model gave lower confidence limits, as much as 1,000 times lower than the multi-hit. These observations led the Council to conclude that the low-dose linearity embodied in the Armitage-Doll model was inappropriate for practical risk estimation. The rationale for this conclusion was apparently that the multi-hit model was more influenced by the shape of the dose-response curve in the high-dose region, which influences the maximum likelihood

estimates in the multistage model but does not strongly influence the confidence limits.

Brown (260) used both hypothetical and experimental data in his comparisons. His initial comparison of the lognormal, logistic, single-hit, multi-hit, Weibull, and multistage models using hypothetical data showed that inordinately large data sets would be needed to conclude which of these models provided the best fit. This observation was confirmed when these models were applied to data on the induction of liver tumors in mice by DDT. In addition, at an excess risk of 10^{-6}, the ranking of VSDs for the various models was as follows: single-hit $\leq$ multistage $<$ Weibull $\leq$ multi-hit $\leq$ logistic $<$ lognormal. The range of estimates covered 6 orders of magnitude and the divergence of the VSDs increased as the excess risk level decreased. Brown also used the data from the "megamouse" study conducted with 2-acetylaminofluorene (261) to determine the effect of including the results from large experimental groups tested at low doses on the model-to-model variation in the VSDs. His conclusion was that the additional data had little effect on the VSD point estimates for the multistage and lognormal models, and more importantly, had little effect on the relative difference between the VSDs estimated from the two models.

Crump et al. (89) used the data from an experiment on thyroid tumor induction in rats with ethylenethiourea (262). Comparing the 95% lower confidence limits of the VSDs at a

risk level of 10^{-6}, these authors found that the one-hit and linear models of Hoel et al. (220) and Gaylor and Kodell (240) yielded the lowest values. The next lowest values were derived by the linear methods of Gross et al. (239), the multistage model, and the log-probit approaches using all the data. The multi-hit model and log-probit method using only the lowest dose level giving a response (221) yielded the largest lower confidence limits. The range of VSD estimates from the different models covered 5 orders of magnitude.

Van Ryzin (258) reviewed the work presented by Krewski and Van Ryzin (263), who compared the models using experimental data. This author noted that with upwardly convex data (19 of the 20 sets examined), the models gave VSDs covering several orders of magnitude in this order: one-hit < multistage < Weibull = logistic = multi-hit < log-probit. With the single downwardly concave data set (vinyl chloride), this order was totally reversed, i.e., the one-hit was the highest, and the log-probit the lowest. The author also noted that this paradox could be resolved if concave data were converted to convex data by accounting for the metabolically effective dose rather than using the administered dose. The applicability of this type of conversion, he stated, requires further experimental investigation.

At the Workshop on Biological and Statistical Implications of the ED_{01} Study and Related Data Bases, results were compiled on analyses of 46 sets of computer-generated bioassay data with time-to-tumor information (264). The data sets were

analyzed by five groups of scientists using five quantal and five time-to-response models. It was found that in the low-dose region point estimates of risk could differ from the actual value by a factor of 1,000 even when precise information on time to tumor was utilized. Linearized upper confidence limits may be very conservative if the true dose-response curve is sublinear in the low-dose range, but when the underlying dose-response curve was actually linear the upper confidence limits did not exceed the actual risk by a factor of more than 10. Krewski et al. (264) found that the value of the additional time-to-tumor information in low-dose extrapolation was to a certain extent questionable in aiding more accurate estimation.

Several conclusions may be drawn from these comparisons. The first is that all the models fit most sets of experimental data almost equally well and that a great deal of data would be needed to determine which provided the statistically best fit. Second, the testing of large numbers of animals at low doses does not produce greater differences among the VSDs generated by the models, nor does it allow for the calculation of VSDs with substantially smaller confidence limits. A third conclusion, really an observation, is that in almost all cases VSD estimates from the various models for a given data set cover several orders of magnitude and fall in the order: one-hit < multistage < Weibull = logistic = multi-hit < log-probit. In rough terms, then, the one-hit model tends to yield the most conservative estimates and the log-probit model the least.

Furthermore, the range of the VSD estimates and the differences in the widths of their confidence limits are due to the mathematical differences between the models, i.e., a confidence limit for a given model fit to a particular data set indicates statistical certainty given the model is correct, but does not measure the model's biological accuracy.

Crump (265) has presented an approach to designing future bioassays so that they would have optimum power for discriminating between preselected possible dose-response models. A computer simulation showed, however, that even with optimally designed large experiments it was difficult to distinguish between the multistage and log-normal models, which had been chosen as an example. This disappointing result indicates that even if definition of a particular dose-response model were adopted as the primary goal of bioassays and they were designed according to optimal principles, they would not be very successful in realizing this objective. Crump concluded that goodness-of-fit of a model to the observed data therefore should not be the sole criterion upon which the selection of a dose-response model is based.

f. Wasted dose

The concept of a "wasted dose" is that a tumor is usually initiated fairly early in the period of exposure and that continued exposure does not contribute any further to its initiation (although it may contribute to its development or growth through later stages). Hence, any dose beyond that necessary to initiate

a tumor is "wasted" in that it was not necessary to initiate the cancer. In practice, however, the actual effective dose is not measured, and dose-response curves are necessarily based on the total doses received by the animal, thereby underestimating the true response per unit dose. The minimum effective dose would have both a dose level component and a time or duration component, much like the time-weighted average calculations used to express exposure to toxic substances. The primary practical limitation in determining a minimum effective dose in animal experiments is that a serial sacrifice design would be needed to identify the initial precancerous lesion. Such a schedule would require many animals; in addition, the histopathology of precancerous lesions is not well known. In epidemiologic studies, if exposures were well defined and there were a short period of time between the initiation of cancer and diagnosis of a tumor, identification of the actual effective dose might be possible, but still highly impractical. As yet, no models explicitly account for the wasted dose concept. Time-to-tumor models are conceptually the closest, although the period of time of concern for determination of the wasted dose is the time between the initial exposure and the occurrence of an irreversible cell or damage to a tissue that would eventually lead to tumor formation. The models of Crump and Howe (123) allow calculation of the effect of exposure at various periods within the lifetime, and therefore permit calculations

of the effects of early and late exposures, within the context and assumptions of the multistage model.

5. Considerations in Extrapolating from Human Data

The previous sections of this chapter were primarily concerned with low-dose extrapolation of data obtained in bioassays with experimental animals. Although the biological principles involved in extrapolating from data obtained from human epidemiologic studies are essentially the same, the practical problems are usually very much smaller, because the extrapolation is usually over a limited range in dose. In most cases, the epidemiologic data will have been obtained from studies of people exposed in the past, and the problem is one of estimating the risks to people exposed to the same substance under present-day conditions and under projected future conditions. Thus, instead of extrapolating over 4-6 orders of magnitude in dose, as is often necessary with animal data, it is usually necessary to extrapolate over only 1, or at most 2, orders of magnitude.

Within this range of exposure, there is much evidence that multistage and Weibull models can be fitted to human dose-response data, at least for the limited number of carcinogens for which numerical data exist (121, 199, 242). Indeed, linear dose-response models have been used for extrapolating human incidence data (199, 258, 259, 266) with little or none of the controversy that attends their application to animal data.

The major problem in use of human data is taking proper account of the timing of exposure and of latency. In most

occupational epidemiologic studies, the groups of workers studied are exposed for a limited period of their working lives and are then followed up for periods that rarely exceed 30 years. Since cancer incidence increases with the duration of exposure and rapidly with age and duration of follow-up, occupational studies usually only detect a fraction of the lifetime risks resulting from exposure. The problem is to estimate full-lifetime risks from partial-lifetime data.

To adjust, first, for differences between groups in the intensity and duration of exposure, it is usually considered appropriate to assume a linear relationship between response and cumulative exposure. That is, response is assumed to be linearly proportional to both the average exposure level and to the duration of exposure (72). To correct for "wasted dose," an appropriate modification of this procedure would be to assume that response is proportional to duration of exposure only up to some maximum duration (either 25 years after first exposure, or 20 years prior to death) (199). A more formal procedure is to use one of the time-to-tumor models such as the Weibull or multistage models (121, 199, 258).

Adjustments for differences in duration of followup are more difficult. Day and Brown (121) have shown that data on human cancers can be fitted to multistage models. Hence, while exposure continues, age-specific incidence continues to increase as a power function (typically T^4) of the elapsed time since initial exposure. In these circumstances, full-lifetime risks

can be estimated by applying this power function to the observed data (72, 89). However, when exposure ceases (as it does when workers change jobs or retire), both empirical data and the multistage model indicate a variety of responses. For early-stage carcinogens, age-specific risks continue to increase with time in a fashion that reflects prior cumulative exposure. However, for late-stage carcinogens, excess risks disappear fairly rapidly after cessation of exposure (121). An example of the use of the multistage model to analyze human data is the assessment of occupational arsenic exposure by Brown and Chu (267). Accordingly, extrapolation procedures should ideally reflect knowledge of the stage at which a carcinogen acts. For early-stage carcinogens, full-lifetime risks can be estimated by the power-function procedure mentioned above. An equivalent procedure, which avoids assumptions about the magnitude of the exponent of the power function, is to assume that relative risk (age-specific incidence in exposed workers divided by that in the general population) is independent of age; then the full-lifetime risk can be estimated by multiplying the observed relative risk by the lifetime incidence in the general population (199, 266). For late-stage carcinogens, full lifetime risks must be estimated by integrating the multistage function, which describes the dependence of incidence on duration of exposure over the distribution of exposure periods (121, 123).

Substantial uncertainty can arise because for many carcinogens the stage of action is not known. When a carcinogen is

known to act at an early stage, or is suspected to do so (e.g. because it is known to be genotoxic), it is appropriate to apply the early-stage model. When a carcinogen is suspected to act at a late stage (e.g., because it is known to be nongenotoxic), it is appropriate to apply the late-stage model. However, some nongenotoxic carcinogens appear to act at an early stage (121). Hence, unless a carcinogen is known to act at a late stage (e.g., from observation that risk declines after cessation of exposure), it is desirable to apply both models even to nongenotoxic carcinogens and to present both results as a range of possible risks.

V. RISK ASSESSMENT FOR OCCUPATIONAL EXPOSURE TO MONOHALOMETHANES

A. Introduction

In this chapter of the report, we derive estimates of the likely magnitude of carcinogenic risks that might accrue to workers occupationally exposed to monohalomethanes. For methyl chloride, the assessment is based on the data on carcinogenicity and mutagenicity that were reviewed in Chapter II, and takes into account the data on metabolism and pharmacokinetics that were reviewed in Chapter III. This information is used to select appropriate scaling factors and dose-response models from among those discussed in Chapter IV, and hence, to derive estimates of risks to humans exposed at various levels and on various schedules. For methyl bromide and methyl iodide, no usable data are available on dose-response relationships, but rough estimates of the magnitude of human risks can be derived using data on relative biological activities and likely mechanisms of action. Uncertainties in risk estimates are discussed in Chapter VI.

To illustrate the dependence of risk upon the circumstances of exposure, a wide range of exposure considerations is considered. First, risk estimates are presented for a wide range of average workplace concentrations (0.5 to 1,000 ppm for methyl chloride). Second, risk estimates are presented for two lengths of working lifetime: 45 years (assumed to be from age 20 to 65) and 54 years (assumed to be from age 16 to 70). Third,

risk estimates are presented for a range of partial exposures (averaging 6, 12, 18, 24, 30 and 40 hours per week). The exposure period of 30 hours per week is the same as that of the mice in the CIIT study (14), whereas that of 40 hours per week is calculated for workers who might be exposed for 8 hours per day, 5 days per week. Because of breaks and down time, it is not clear whether 30 or 40 hours per week is the more appropriate value to use for "full-time" exposure. The exposure periods of 6, 12, 18 and 24 hours per week are included to illustrate a range of partial exposures (e.g., those resulting from jobs with intermittent exposure). For purposes of calculation, it is assumed that these partial exposures are distributed uniformly through the working lifetime. As discussed in Section IV.B.5, carcinogenic risks may depend strongly on the schedule of exposure within the working lifetime; for example, a period of exposure to a carcinogenic initiator early in life is expected to lead to much higher risks than the same exposure late in life. These effects are not explicitly evaluated here, but can be calculated from the tables in reference 123. The possible dependence of carcinogenic risks on intermittency and variability in exposure within a working day or week is also not considered. This is equivalent to the assumption that the carcinogenic risk arising from fluctuating exposure to monohalomethanes is the same as would arise from continuous exposure to the time-weighted average concentration.

B. Methyl Chloride

1. Dose-Response Data and Mechanisms of Action

Dose-response data for the carcinogenic effects of methyl chloride have been summarized in Sections II.A.4 and II.F.1 of this report. The data summarized in Tables II-3 and II-4 provide direct measures of the dose-response relationship for induction of kidney tumors in male mice. These are the only useful dose-response data for methyl chloride and are used as the basis for the risk assessment that follows.

Methyl chloride is a direct-acting alkylating agent, a direct-acting mutagen, and possibly an indirect-acting mutagen. There is no evidence that it induces cancer in mice by an indirect mechanism or that its dose-response curve is markedly nonlinear (Section II.E.1). Accordingly, it is reasonable to assume that it is likely to be a carcinogenic initiator and to use a model of the dose-response relationship that is consistent with low-dose linearity (Sections IV.C.2 and IV.C.4). Data on metabolism and pharmacokinetics of methyl chloride provide no substantial evidence of nonlinear kinetics (Section III.E), so that it is not necessary to modify the dose-response relationship to account for a nonlinear relationship between target tissue dose and external exposure. If anything, this assumption may underestimate the risks posed by methyl chloride at low doses, since one experiment indicated that the concentration of nonextractable radioactivity in the kidney increased

less rapidly than in proportion to the external concentration of radiolabeled methyl chloride (Figure III-1).

For the purposes of this risk assessment, data on both benign and malignant tumors (Tables II-3 and II-4) are used as the basis for estimating carcinogenic response. Although the stages of development of kidney tumors in mice are not well documented, the mice studied in reference 6 showed a spectrum of tumor development that included both benign and malignant tumors of the same cell type, as well as related pre-neoplastic lesions. Thus, it seems reasonable to treat both benign and malignant tumors together as measures of carcinogenic response. If only malignant tumors (Table II-3) were considered in risk assessment, estimates of risks would be reduced by a factor of about 3.

2. Scaling Factors

To estimate human risks, it will be assumed that exposed workers would be subjected to the same risks as male mice when exposed to the same ambient concentrations of methyl chloride for the same fractions of their lifetimes. This incorporates two separate assumptions: (i) that the appropriate scaling factor for dose between species is the ambient concentration in ppm; (ii) that workers are as susceptible as male mice when exposed to the same (appropriately scaled) lifetime pattern of dosage. The first assumption conforms to current practice (72) and appears reasonable in the light of the discussion in Section IV.B.2, although its empirical basis is limited.

The second assumption is made in the absence of any specific evidence to assume greater or lesser susceptibility. The meager data on metabolism and pharmacokinetics of methyl chloride show no evidence of differences between humans and animals that would justify any adjustment of effective doses, nor any evidence that would justify the assumption of any unique susceptibility of male mice. Empirical data on other carcinogens provide very limited support for an assumption of equal susceptibility of humans and rodents (Section IV.B.2). The assumption that workers would be as susceptible as male mice, the most susceptible of the four species-sex groups tested in reference 14, is made conservatively, in the absence of any scientific basis for assuming lesser susceptibility.

For purposes of calculation, we use 72 years as the mean lifetime of humans, and assume that this is equivalent to 24 months in B6C3F1 mice. We recognize that some humans live much longer than 72 years and that some mice live much longer than 24 months. However, in both species, competing causes of death become dominant beyond these ages and the calculation of cancer risks is severely complicated by this fact. Our calculation of "lifetime" risk in a human population is intended to represent the excess risk of developing cancer through age 72 among individuals who survive to that age.

No assumption is made in this report about the likely site of action of methyl chloride in humans. Pharmacokinetic data indicate that the chemical is retained in the human body

for long enough to be circulated to all potential target organs (45, 46). There is little basis for assuming that the target organ in humans would be the same as in mice (41).

3. Selection of Dose-Response Models

For the risk assessment that follows, the multistage and Weibull models are selected for extrapolation of the empirical data to lower exposure levels. This selection is based on the information cited in Section V.B.1 above. Among the other dose-response models discussed in Chapter IV, the log-probit model is not used because of its lack of a biological basis and exclusion of low-dose linearity; the gamma multi-hit model is not used because methyl chloride is a direct-acting mutagen for which a multi-hit mechanism is implausible, and because the observed dose-response relationship is not markedly nonlinear; the one-hit and other linear models are not used because data on methyl chloride are available from several dose levels; and pharmacokinetic models are not used because no useful data are available on pharmacokinetics or adduct-formation that would permit their use.

The multistage model is selected as the most appropriate model for application to the dichotomous data in Table II-4, whereas the Weibull model is selected as the most appropriate model for application to the time-to-tumor data in Table II-3. The advantages of the multistage model are that it is based on a biological mechanism consistent with current understanding of the process of carcinogenesis, and that it is directly

applicable to direct-acting substances such as methyl chloride, which have dose-response relationships which are likely to be linear at low doses. Its disadvantage in this case is that the dichotomous data tabulated in Table II-4 include only part of the information generated in the experiment. The advantages of the Weibull model are that it uses all the data in Table II-3 and that it is consistent with a wide range of dose-response relationships. Its disadvantages are that it is phenomenological rather than biologically based (although it is mathematically related both to the multistage and multi-hit models) and that its use requires an assumption that the data used represent an unbiased set of data on the time of occurrence of tumors; this assumption is uncertain because there is no information whether the tumors were the cause of death of any of the mice. The advantages and disadvantages of each model are complementary, and the use of both models should provide information about the range of reasonable estimates of risk that are consistent with the data. (However, it should be emphasized that other, less plausible, models would yield much wider ranges of risk estimates.)

4. Risk Estimates from the Multistage Model

The multistage model is of the form

$$P(d) = 1 - \exp(-\Sigma q_i d^i)$$

where P(d) is the probability of developing cancer after lifetime exposure to dose d, and the q_i are nonnegative coefficients to be estimated from the data. This model was fitted to the

data on the second line of Table II-4, using a computer program developed by Howe and Crump (268). This program yields two sets of estimates of the model parameters. The best fit of the model to the data, or maximum likelihood estimate (MLE), included only a third-order term in dose, and the MLE estimate of the coefficient q_3 was 2.6×10^{-10} $(ppm)^{-3}$. The 95th percentile upper confidence limit (UCL) on the dose-response relationship included a first-order term, and the UCL estimate of the coefficient q_1^* was 2.7×10^{-4} $(ppm)^{-1}$. These two terms dominate estimates of risk over the entire dose range under consideration.

For application to human risk assessment, these estimates of the form of the dose-response relationship require several adjustments. First, the data in Table II-4 refer only to mice dying through the 21st month of observation. To estimate lifetime risks to mice (i.e., risks through the 24th month), we follow the procedures of references 72 and 89 and increase the risks estimated by the model by a factor of $(24/21)^4$, or 1.71. This is equivalent to increasing the coefficients q_3 and q_1^* by the factor 1.71, or to decreasing the effective doses by factors of 1.19 in the MLE model and 1.71 in the UCL model (82).

Second, the adjusted data refer to mice exposed to a constant concentration of methyl chloride throughout life. To estimate the risks to workers exposed for part of their lifetimes, we used formulae computed by Crump and Howe (123) from the multistage model. For this purpose, we assumed that methyl

chloride would act at the first stage of a 5-stage carcinogenic process. For constant exposure from age 20 to 65, Crump and Howe's formulae then indicate a lifetime risk 0.212 times that for lifetime exposure to the same concentration; for constant exposure from ages 16 to 70, the corresponding ratio is 0.301.

Finally, the effective doses were multiplied by factors of 0.2., 0.4, 0.6, 0.8, and 1.0 to correspond to the assumed exposures for 20, 40, 60, 80, and 100% of the working period.

After these adjustments were made to the effective doses and risks, the relationships between risks and exposure patterns were calculated for the various circumstances of exposure listed in Section V.A. Tables V-1 and V-2 present estimates of risks for six widely spaced exposure concentrations under 10 assumptions about the duration and intermittency of exposure. Tables V-3 and V-4 present estimates of the exposure levels that correspond to five widely spaced risk levels under the same 10 assumptions about exposure. In each table, both the MLE and 95th percentile confidence limits (UCL for risks, lower confidence limit (LCL) for exposure levels) are presented.

5. Risk Estimates from the Weibull Model

The Weibull model is of the form

$$P(t,d) = 1 - \exp[-(t-t_o)^k \Sigma q_i d^i],$$

where $P(t,d)$ is the incremental risk of cancer at time t and dose d, and t_o, k and q_i are nonnegative constants to be estimated from the data. This model was fitted to the data in Table II-3, using a computer program developed by Howe and

TABLE V-1
ESTIMATED RISK AT FIXED EXPOSURE LEVELS FOR
METHYL CHLORIDE BASED ON THE MULTISTAGE MODEL

WORKING LIFETIME OF 45 YEARS

Exposure Level (ppm)		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
0.5	MLE[a]	4×10^{-13}	1×10^{-12}	3×10^{-12}	6×10^{-12}	1×10^{-11}	3×10^{-11}
	UCL[b]	1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}	5×10^{-5}	6×10^{-5}
5.0	MLE	9×10^{-11}	8×10^{-10}	3×10^{-9}	6×10^{-9}	1×10^{-8}	3×10^{-8}
	UCL	1×10^{-4}	2×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	6×10^{-4}
25	MLE	1×10^{-8}	9×10^{-8}	3×10^{-7}	8×10^{-7}	1×10^{-6}	3×10^{-6}
	UCL	5×10^{-4}	1×10^{-3}	1×10^{-3}	2×10^{-3}	2×10^{-3}	3×10^{-3}
100	MLE	8×10^{-7}	6×10^{-6}	2×10^{-5}	5×10^{-5}	9×10^{-5}	2×10^{-4}
	UCL	2×10^{-3}	4×10^{-3}	6×10^{-3}	8×10^{-3}	9×10^{-3}	1×10^{-2}
500	MLE	9×10^{-5}	8×10^{-4}	3×10^{-3}	6×10^{-3}	1×10^{-2}	3×10^{-2}
	UCL	9×10^{-3}	2×10^{-2}	3×10^{-2}	4×10^{-2}	4×10^{-2}	6×10^{-2}
1000	MLE	8×10^{-4}	6×10^{-3}	2×10^{-2}	4×10^{-2}	8×10^{-2}	1×10^{-1}
	UCL	2×10^{-2}	4×10^{-2}	5×10^{-2}	6×10^{-2}	8×10^{-2}	1×10^{-1}

[a]Maximum likelihood estimate

[b]95th percentile upper confidence limit on risk

TABLE V-2
ESTIMATED RISK AT FIXED EXPOSURE LEVELS FOR
METHYL CHLORIDE BASED ON THE MULTISTAGE MODEL

WORKING LIFETIME OF 54 YEARS

Exposure Level (ppm)		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
0.5	MLE[a]	6×10^{-13}	2×10^{-12}	4×10^{-12}	9×10^{-12}	2×10^{-11}	4×10^{-11}
	UCL[b]	1×10^{-5}	3×10^{-5}	4×10^{-5}	5×10^{-5}	7×10^{-5}	9×10^{-5}
5.0	MLE	1×10^{-10}	1×10^{-9}	4×10^{-9}	9×10^{-9}	2×10^{-8}	4×10^{-8}
	UCL	1×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	7×10^{-4}	9×10^{-4}
25	MLE	2×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	2×10^{-6}	5×10^{-6}
	UCL	7×10^{-4}	1×10^{-3}	2×10^{-3}	3×10^{-3}	3×10^{-3}	5×10^{-3}
100	MLE	1×10^{-6}	9×10^{-6}	3×10^{-5}	7×10^{-5}	1×10^{-4}	3×10^{-4}
	UCL	3×10^{-3}	5×10^{-3}	8×10^{-3}	1×10^{-2}	1×10^{-2}	2×10^{-2}
500	MLE	1×10^{-4}	1×10^{-3}	4×10^{-3}	8×10^{-3}	2×10^{-2}	4×10^{-2}
	UCL	1×10^{-2}	3×10^{-2}	4×10^{-2}	5×10^{-2}	6×10^{-2}	8×10^{-2}
1000	MLE	1×10^{-3}	8×10^{-3}	3×10^{-2}	6×10^{-2}	1×10^{-1}	2×10^{-1}
	UCL	3×10^{-2}	5×10^{-2}	7×10^{-2}	9×10^{-2}	1×10^{-1}	1×10^{-1}

[a]Maximum likelihood estimate

[b]95th percentile upper confidence limit on risk

TABLE V-3
ESTIMATED EXPOSURE LEVEL AT FIXED RISK FOR
METHYL CHLORIDE BASED ON THE MULTISTAGE MODEL[a]

WORKING LIFETIME OF 45 YEARS

Risk		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
10^{-2}	MLE[b]	2×10^{3}	1×10^{3}	8×10^{2}	6×10^{2}	5×10^{2}	4×10^{2}
	LCL[c]	5×10^{2}	3×10^{2}	2×10^{2}	1×10^{2}	1×10^{2}	8×10^{1}
10^{-3}	MLE	1×10^{3}	5×10^{2}	4×10^{2}	3×10^{2}	2×10^{2}	2×10^{2}
	LCL	5×10^{1}	3×10^{1}	2×10^{1}	1×10^{1}	1×10^{1}	8×10^{0}
10^{-4}	MLE	5×10^{2}	3×10^{2}	2×10^{2}	1×10^{2}	1×10^{2}	8×10^{1}
	LCL	5×10^{0}	3×10^{0}	2×10^{0}	1×10^{0}	1×10^{0}	8×10^{-1}
10^{-5}	MLE	2×10^{2}	1×10^{2}	8×10^{1}	6×10^{1}	5×10^{1}	4×10^{1}
	LCL	5×10^{-1}	3×10^{-1}	2×10^{-1}	1×10^{-1}	1×10^{-1}	8×10^{-2}
10^{-6}	MLE	1×10^{2}	5×10^{1}	4×10^{1}	3×10^{1}	2×10^{1}	2×10^{1}
	LCL	5×10^{-2}	3×10^{-2}	2×10^{-2}	1×10^{-2}	1×10^{-2}	8×10^{-3}

[a]Exposure levels expressed in ppm

[b]Maximum likelihood estimate

[c]95th percentile lower confidence limit on dose

TABLE V-4
ESTIMATED EXPOSURE LEVEL AT FIXED RISK FOR
METHYL CHLORIDE BASED ON THE MULTISTAGE MODEL[a]

WORKING LIFETIME OF 54 YEARS

Risk		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
10^{-2}	MLE[b]	$2x10^{3}$	$1x10^{3}$	$7x10^{2}$	$5x10^{2}$	$4x10^{2}$	$3x10^{2}$
	LCL[c]	$4x10^{2}$	$2x10^{2}$	$1x10^{2}$	$9x10^{1}$	$7x10^{1}$	$6x10^{1}$
10^{-3}	MLE	$1x10^{3}$	$5x10^{2}$	$3x10^{2}$	$2x10^{2}$	$2x10^{2}$	$1x10^{2}$
	LCL	$4x10^{1}$	$2x10^{1}$	$1x10^{1}$	$9x10^{0}$	$7x10^{0}$	$5x10^{0}$
10^{-4}	MLE	$5x10^{2}$	$2x10^{2}$	$2x10^{2}$	$1x10^{2}$	$9x10^{1}$	$7x10^{1}$
	LCL	$4x10^{0}$	$2x10^{0}$	$1x10^{0}$	$9x10^{-1}$	$7x10^{-1}$	$5x10^{-1}$
10^{-5}	MLE	$2x10^{2}$	$1x10^{2}$	$7x10^{1}$	$5x10^{1}$	$4x10^{1}$	$3x10^{1}$
	LCL	$4x10^{-1}$	$2x10^{-1}$	$1x10^{-1}$	$9x10^{-2}$	$7x10^{-2}$	$5x10^{-2}$
10^{-6}	MLE	$1x10^{2}$	$5x10^{1}$	$3x10^{1}$	$2x10^{1}$	$2x10^{1}$	$1x10^{1}$
	LCL	$4x10^{-2}$	$2x10^{-2}$	$1x10^{-2}$	$9x10^{-3}$	$7x10^{-3}$	$5x10^{-3}$

[a]Exposure levels expressed in ppm

[b]Maximum likelihood estimate

[c]95th percentile lower confidence limit on dose

Crump (269). This program also yields two sets of estimates of the model parameters. For both sets, the estimates of t_o and k were 10.56 months and 1.116 respectively. The MLE, or best fit of the model to the data, yielded two non-zero coefficients, $q_1 = 4.0 \times 10^{-6}$ $(ppm)^{-1}$ and $q_2 = 2.1 \times 10^{-8}$ $(ppm)^{-2}$. The UCL, or 95th percentile upper confidence limit on the dose-response relationship, was dominated by the first-order term, for which the coefficient q_1^* was 4.2×10^{-4} $(ppm)^{-1}$.

For risk assessment, the fitted models were used to estimate cumulative risks to 24 months (assumed to be a mouse lifetime). Since the Weibull model is not based on an explicit model of the development of cancer, no specific formulae are available to compute the relationships between risks resulting from full-lifetime and partial-lifetime exposures. To estimate the likely magnitude of risks to workers exposed for part of their lifetimes, we assumed that risks would be proportional to cumulative exposure. However, to eliminate "wasted doses" (see Section IV.C.4.f), we considered for this estimate exposures only through age 55. Hence, workers exposed from age 20 onwards would incur a risk 35/55 or 0.64 times that resulting from lifetime exposure, whereas for workers exposed from age 16 onwards the corresponding ratio would be 39/55 or 0.71. These factors were applied to the lifetime risks calculated from the model. In addition, the average exposure concentration was adjusted for partial exposure by multiplying by the appropriate factors (0.2, 0.4, 0.6, 0.8, and 1.0).

The resulting estimates of relationships between risks and exposure patterns were calculated for the various circumstances of exposure listed in Section V.A. Tables V-5 and V-6 present estimates of risks for the same 60 exposure circumstances as those considered in Tables V-1 and V-2. Tables V-7 and V-8 present estimates of the exposure levels that correspond to the same risk levels as those considered in Tables V-3 and V-4. In each table, both the MLE and 95th percentile confidence limits are presented.

All estimates presented in Tables V-1 to V-8 are rounded to one significant figure, because of the wide range of uncertainties in the estimates. These uncertainties are discussed in Chapter VI.

C. Methyl Bromide and Methyl Iodide

As discussed in Sections II.F.2 and II.F.3, the only useful data on dose-response relationships for methyl bromide and methyl iodide are measurements of relative biological activity in in vitro assays for mutagenicity. The most directly comparable data are those provided in references 25 and 26, in which all three monohalomethanes were tested in similar conditions in the Ames assay. These indicated that the relative mutagenic potencies of methyl chloride, methyl bromide, and methyl iodide were approximately in the ratios 1:40:10 (see Section II.D.1). The greater biological activity of methyl bromide than methyl iodide in this assay agrees with the data on their relative reactivities with nucleophilic compounds where there is a similar

TABLE V-5
ESTIMATED RISK AT FIXED EXPOSURE LEVELS FOR
METHYL CHLORIDE BASED ON THE WEIBULL MODEL

WORKING LIFETIME OF 45 YEARS

Exposure Level (ppm)		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
0.5	MLE[a]	$5x10^{-6}$	$9x10^{-6}$	$1x10^{-5}$	$2x10^{-5}$	$2x10^{-5}$	$3x10^{-5}$
	UCL[b]	$3x10^{-5}$	$5x10^{-5}$	$8x10^{-5}$	$1x10^{-4}$	$1x10^{-4}$	$2x10^{-4}$
5.0	MLE	$5x10^{-5}$	$9x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$	$2x10^{-4}$	$3x10^{-4}$
	UCL	$3x10^{-4}$	$5x10^{-4}$	$8x10^{-4}$	$1x10^{-3}$	$1x10^{-3}$	$2x10^{-3}$
25	MLE	$2x10^{-4}$	$5x10^{-4}$	$7x10^{-4}$	$1x10^{-3}$	$1x10^{-3}$	$2x10^{-3}$
	UCL	$1x10^{-3}$	$3x10^{-3}$	$4x10^{-3}$	$5x10^{-3}$	$7x10^{-3}$	$9x10^{-3}$
100	MLE	$1x10^{-3}$	$2x10^{-3}$	$4x10^{-3}$	$5x10^{-3}$	$7x10^{-3}$	$1x10^{-2}$
	UCL	$5x10^{-3}$	$1x10^{-2}$	$2x10^{-2}$	$2x10^{-2}$	$3x10^{-2}$	$3x10^{-2}$
500	MLE	$7x10^{-3}$	$2x10^{-2}$	$3x10^{-2}$	$5x10^{-2}$	$8x10^{-2}$	$1x10^{-1}$
	UCL	$3x10^{-2}$	$5x10^{-2}$	$7x10^{-2}$	$1x10^{-1}$	$1x10^{-1}$	$2x10^{-1}$
1000	MLE	$2x10^{-2}$	$5x10^{-2}$	$1x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$	$3x10^{-1}$
	UCL	$5x10^{-2}$	$1x10^{-1}$	$1x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$	$3x10^{-1}$

[a]Maximum likelihood estimate

[b]95th percentile upper confidence limit on risk

TABLE V-6
ESTIMATED RISK AT FIXED EXPOSURE LEVELS FOR
METHYL CHLORIDE BASED ON THE WEIBULL MODEL

WORKING LIFETIME OF 54 YEARS

Exposure Level (ppm)		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
0.5	MLE[a]	$5x10^{-6}$	$1x10^{-5}$	$2x10^{-5}$	$2x10^{-5}$	$3x10^{-5}$	$3x10^{-5}$
	UCL[b]	$3x10^{-5}$	$6x10^{-5}$	$9x10^{-5}$	$1x10^{-4}$	$1x10^{-4}$	$2x10^{-4}$
5.0	MLE	$5x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$	$2x10^{-4}$	$3x10^{-4}$	$4x10^{-4}$
	UCL	$3x10^{-4}$	$6x10^{-4}$	$9x10^{-4}$	$1x10^{-3}$	$1x10^{-3}$	$2x10^{-3}$
25	MLE	$3x10^{-4}$	$5x10^{-4}$	$8x10^{-4}$	$1x10^{-3}$	$1x10^{-3}$	$2x10^{-3}$
	UCL	$1x10^{-3}$	$3x10^{-3}$	$4x10^{-3}$	$6x10^{-3}$	$7x10^{-3}$	$1x10^{-2}$
100	MLE	$1x10^{-3}$	$2x10^{-3}$	$4x10^{-3}$	$6x10^{-3}$	$8x10^{-3}$	$1x10^{-2}$
	UCL	$6x10^{-3}$	$1x10^{-2}$	$2x10^{-2}$	$2x10^{-2}$	$3x10^{-2}$	$4x10^{-2}$
500	MLE	$8x10^{-3}$	$2x10^{-2}$	$4x10^{-2}$	$6x10^{-2}$	$8x10^{-2}$	$1x10^{-1}$
	UCL	$3x10^{-2}$	$6x10^{-2}$	$8x10^{-2}$	$1x10^{-1}$	$1x10^{-1}$	$2x10^{-1}$
1000	MLE	$2x10^{-2}$	$6x10^{-2}$	$1x10^{-1}$	$2x10^{-1}$	$3x10^{-1}$	$4x10^{-1}$
	UCL	$6x10^{-2}$	$1x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$	$3x10^{-1}$

[a]Maximum likelihood estimate

[b]95th percentile upper confidence limit on risk

TABLE V-7
ESTIMATED EXPOSURE LEVEL AT FIXED RISK FOR
METHYL CHLORIDE BASED ON THE WEIBULL MODEL[a]

WORKING LIFETIME OF 45 YEARS

Risk		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
10^{-2}	MLE[b]	$7x10^{2}$	$3x10^{2}$	$2x10^{2}$	$2x10^{2}$	$1x10^{2}$	$1x10^{2}$
	LCL[c]	$2x10^{2}$	$1x10^{2}$	$6x10^{1}$	$5x10^{1}$	$4x10^{1}$	$3x10^{1}$
10^{-3}	MLE	$1x10^{2}$	$5x10^{1}$	$3x10^{1}$	$2x10^{1}$	$2x10^{1}$	$1x10^{1}$
	LCL	$2x10^{1}$	$9x10^{0}$	$6x10^{0}$	$5x10^{0}$	$4x10^{0}$	$3x10^{0}$
10^{-4}	MLE	$1x10^{1}$	$5x10^{0}$	$4x10^{0}$	$3x10^{0}$	$2x10^{0}$	$2x10^{0}$
	LCL	$2x10^{0}$	$9x10^{-1}$	$6x10^{-1}$	$5x10^{-1}$	$4x10^{-1}$	$3x10^{-1}$
10^{-5}	MLE	$1x10^{0}$	$5x10^{-1}$	$4x10^{-1}$	$3x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$
	LCL	$2x10^{-1}$	$9x10^{-2}$	$6x10^{-2}$	$5x10^{-2}$	$4x10^{-2}$	$3x10^{-2}$
10^{-6}	MLE	$1x10^{-1}$	$5x10^{-2}$	$4x10^{-2}$	$3x10^{-2}$	$2x10^{-2}$	$2x10^{-2}$
	LCL	$2x10^{-2}$	$9x10^{-3}$	$6x10^{-3}$	$5x10^{-3}$	$4x10^{-3}$	$3x10^{-3}$

[a]Exposure levels expressed in ppm

[b]Maximum likelihood estimate

[c]95th percentile lower confidence limit on dose

TABLE V-8
ESTIMATED EXPOSURE LEVEL AT FIXED RISK FOR
METHYL CHLORIDE BASED ON THE WEIBULL MODEL[a]

WORKING LIFETIME OF 54 YEARS

Risk		Hours Worked Weekly for a Lifetime					
		6	12	18	24	30	40
10^{-2}	MLE[b]	$6x10^{2}$	$3x10^{2}$	$2x10^{2}$	$2x10^{2}$	$1x10^{2}$	$9x10^{1}$
	LCL[c]	$2x10^{2}$	$9x10^{1}$	$6x10^{1}$	$4x10^{1}$	$3x10^{1}$	$3x10^{1}$
10^{-3}	MLE	$9x10^{1}$	$4x10^{1}$	$3x10^{1}$	$2x10^{1}$	$2x10^{1}$	$1x10^{1}$
	LCL	$2x10^{0}$	$8x10^{0}$	$6x10^{0}$	$4x10^{0}$	$3x10^{0}$	$3x10^{0}$
10^{-4}	MLE	$1x10^{1}$	$5x10^{0}$	$3x10^{0}$	$2x10^{0}$	$2x10^{0}$	$1x10^{0}$
	LCL	$2x10^{0}$	$8x10^{-1}$	$6x10^{-1}$	$4x10^{-1}$	$3x10^{-1}$	$3x10^{-1}$
10^{-5}	MLE	$1x10^{0}$	$5x10^{-1}$	$3x10^{-1}$	$2x10^{-1}$	$2x10^{-1}$	$1x10^{-1}$
	LCL	$2x10^{-1}$	$8x10^{-2}$	$6x10^{-2}$	$4x10^{-2}$	$3x10^{-2}$	$3x10^{-2}$
10^{-6}	MLE	$1x10^{-2}$	$5x10^{-2}$	$3x10^{-2}$	$2x10^{-2}$	$2x10^{-2}$	$1x10^{-2}$
	LCL	$2x10^{-2}$	$8x10^{-3}$	$6x10^{-3}$	$4x10^{-3}$	$3x10^{-3}$	$3x10^{-3}$

[a]Exposure levels expressed in ppm

[b]Maximum likelihood estimate

[c]95th percentile lower confidence limit on dose

ratio of 1:20:4 (see Section I.A.). Thus, the relative potencies of the three compounds in the Ames assay appear to parallel their activity as alkylating agents, and are consistent with the hypothesis that their mutagenic activity results from their ability to alkylate DNA.

Before this hypothesis can be extended to predict their relative carcinogenic activity, it is necessary to consider whether they may differ also in their ability to reach the DNA in the likely target tissues. Information on the metabolism and pharmacokinetics of the three compounds (reviewed in Chapter III) is limited and no study provides direct comparative data on even two of the compounds. However, various studies indicate that some inhaled methyl chloride is exhaled as methyl chloride, whereas ingested methyl bromide and inhaled methyl iodide are not exhaled in their original form. This implies that methyl bromide and methyl iodide are broken down rapidly in the body and are less likely than methyl chloride to reach distant target organs such as the kidney. This is supported by the study reported in reference 13, which indicated that inhaled methyl bromide does alkylate DNA in liver and spleen cells in vivo, but at a rate much less than expected from in vitro studies.

The data suggest, therefore, that methyl bromide and methyl iodide are less likely than methyl chloride to induce cancer at distant sites in the body and more likely to act at sites of first contact, such as the lung. If so, and if local detoxification processes do not differ among target organs, their

relative activity in initiating cancer is likely to be roughly in proportion to their relative alkylation activity and mutagenic potency, 1:40:10. However, because of the speculative nature of these arguments, it would not be justified to incorporate them into formal risk assessments.

VI. SCIENTIFIC UNCERTAINTIES IN RISK ASSESSMENTS FOR MONOHALOMETHANES

A. Qualitative Inferences of Potential Carcinogenicity

As discussed in Section IV.B.2 of this report, it is an accepted scientific principle that chemicals found to be carcinogenic in animals are likely to be carcinogenic in humans under appropriate conditions of exposure (41, 66-74). However, the strength of this inference depends on the nature of the evidence for carcinogenicity in animals, the similarity of the exposure, and the nature and strength of any countervailing evidence.

Methyl chloride has been found to be carcinogenic in mice in a well-conducted experiment that gave clearly positive results at exposure levels within the range of interest for this risk assessment. It is a direct-acting alkylating agent and a direct-acting mutagen, and there is no evidence that it acts in mice by an indirect mechanism. There is no metabolic or pharmacokinetic evidence that it could be handled differently by humans and mice, and it is known to be retained in the human body for long enough to reach target tissues. This gives rise to a strong inference that it would be carcinogenic in humans in appropriate circumstances. The only countervailing evidence is that the carcinogenic effect was only noted in male mice, and not in female mice or in male or female rats. Because of the general correlation between carcinogenic activity of substances in rats and mice (76, 87, 88), this difference is more likely to be a quantitative difference in susceptibility

than a qualitative difference in response. However, in the absence of scientific understanding of the reasons for the differences, the hypothesis that male mice might be unique in their carcinogenic response to methyl chloride (e.g., through a unique metabolic pathway) cannot be excluded. Hence, the probability that inhaled methyl chloride would be carcinogenic in humans, although high, is less than 100%.

The inference of likely carcinogenicity for the other two monohalomethanes is substantially weaker. There is limited evidence for the carcinogenicity of methyl iodide in rats; both methyl bromide and methyl iodide are mutagenic in appropriate test systems; and methyl bromide is known to alkylate DNA in vitro and in vivo. This constitutes fairly strong evidence for likely carcinogenicity if the chemicals reach target tissues. This potential is greatest at sites of first contact, such as the lung; both compounds appear to be broken down rapidly after absorption into the body. This might reduce or eliminate the likelihood of carcinogenic effects in distant target organs, unless the metabolites were carcinogenic also. Overall, the qualitative inference that methyl bromide and methyl iodide would be carcinogenic in humans is only moderately strong.

B. Quantitative Estimates of Likely Carcinogenic Risks

As discussed in Section V.C, the inferences about the likely carcinogenic potency of methyl bromide and methyl iodide are quite speculative, because it is not known whether and to what extent their greater mutagenic potency would be offset

by their more rapid breakdown. The remainder of this section is limited to discussion of methyl chloride.

1. Uncertainties Arising from Choice of Model

Tables V-1 to V-8 present estimates of risk derived by applying the Weibull and multistage models to different subsets of data from the same experiment. Comparison of these tables shows that the upper confidence limit (UCL) estimates of risk from the two models generally agree within factors of 2-4 throughout the range of exposures considered. Estimates of risk from the Weibull model are generally higher than those from the multistage model for the same exposures, because the correction factors applied to the Weibull estimates for partial lifetime exposure were smaller. However, the maximum likelihood estimates (MLE) from the two models differed much more. The multistage model yielded estimates that were lower by factors ranging from 2 at the highest exposure level (1,000 ppm, 100% exposure factor) to 10^4-10^6 at exposure concentrations of of 5 ppm, and to 10^6-10^7 at exposure concentration of 0.5 ppm. The divergence between the models is greater for smaller percentages of working lifetime exposed. The reason for this wide divergence is that the Weibull model has a linear term in dose, whereas the MLE multistage model has only a third-order term in dose. The reason for this difference is that the subset of data used to fit the multistage model was not matched for age-distributions and, hence, exaggerates the curvature of the dose-response relationship (see Section II.A.4). Thus, at low dose levels,

the MLE multistage model probably underestimates risks and the MLE Weibull model should be preferred. However, even at the relatively high exposure concentration of 100 ppm, the two models diverge by factors of 100 to 1,000, the Weibull model predicting risks greater than 10^{-3} while the multistage model predicts risks less than 10^{-4}.

Although the UCL estimates of risks predicted by the Weibull and multistage models generally agree within factors of 2-4 over a wide range of doses, this does not mean that the estimates are accurate within a factor of 2-4, because the Weibull and multistage models are mathematically similar. Although there are substantial scientific reasons for using these models for methyl chloride (see Section V.B.3), other models are available and could be used if these scientific arguments are not accepted. Comparative studies of these and other models (see Section IV.C.4.e) have shown that most models give similar estimates of risk over the range of risks down to about 10^{-3}, but then the models diverge rapidly, so that their estimates of virtually safe doses differ by several orders of magnitude at a risk levels of 10^{-6}. In this low-dose range, the multistage and Weibull models tend to give "conservative" estimates of risk, i.e., higher estimates at a given dose level than some other models. Thus, the estimates presented in Tables V.1 to V.8 are not strongly model-dependent at risk levels above 10^{-3}, but become increasingly model-dependent at lower risk levels, and are extremely uncertain at risk levels below 10^{-5}.

2. Uncertainties Arising from Different Temporal Patterns of Exposure

The dose-response data derived from the mouse bioassay have been adjusted in several ways to take account of differences between the temporal pattern of exposure of the mice and of occupationally exposed workers. The first adjustment of the multistage data, to estimate lifetime risks from 21-month tumor incidence data, is probably not a major source of uncertainty. The adjustment was biologically reasonable, was by a relatively small multiplicative factor (1.71), and is in fair agreement with the corresponding factor estimated from the data using the Weibull model (1.33). Uncertainties arising from the adjustment for partial-lifetime exposure of the workers are potentially of greater magnitude. Although the adjustment under the multistage model was based on a precise calculation from the model, assumptions were required about the stage of action of methyl chloride. If these assumptions had been wrong, the adjustment factors could have been too low by factors up to 2 or 3 (123). The same range of uncertainty probably arises in the adjustment under the Weibull model, because the assumptions made in this adjustment were similarly arbitrary. The third adjustment, for exposure during only part of the working lifetime, involved the assumption that carcinogenic risk is dependent only on cumulative dose and is independent of short-term fluctuations. This is reasonable for initiation of cancer by chemicals such as methyl chloride, for which there is no evidence of nonlinear pharmacokinetics. However, if methyl chloride also acts at

a later stage in carcinogenesis, ignoring short-term fluctuations in exposure might lead to substantial errors.

3. Uncertainties Arising from Pharmacokinetics and Scaling Factors

The risk assessment presented in Chapter V involved the assumption that ambient concentration in ppm is an equivalent measure of effective dose in mice and in humans. Although a reasonable biological argument can be made for selection of this scaling factor (see Section IV.B.2), the empirical evidence for its selection is scanty. If the correct scaling factor for dose were in fact mg/kg/body weight, the selection of ppm would overestimate human risk by a factor of about 10.

Selection of a scaling factor involves an implicit assumption about relative pharmacokinetics. For example, assuming that concentration in ppm provides an appropriate scaling between species is consistent with the assumption that retention time is proportional to the one-third power of body weight. In the absence of specific comparative data on pharmacokinetics of methyl chloride in humans and mice (see Section III.B), this assumption could be substantially in error.

4. Uncertainties Arising from Differences in Intrinsic Susceptibility

The last major assumption made in the risk assessment is that humans would be as susceptible as male mice to the carcinogenic effects of methyl chloride. In the absence of specific data on the susceptibility of humans to methyl chloride, the only empirical basis for this assumption is the limited

data available on other chemicals. As discussed in Section IV.B.2, these data show a broad correlation between carcinogenic potencies of 13 chemicals in humans and animals, but reveal deviations of up to at least a factor of 10 in each direction (76, 85, 87). An additional complication in the case of methyl chloride is that the animal data are internally inconsistent: male mice are at least an order of magnitude more susceptible than female mice or male or female rats. If humans were in fact equal in susceptibility to female mice, rather than male mice, all the estimates of risk in Tables V-1 to V-8 would be too high by at least an order of magnitude.

5. Summary

The largest sources of uncertainty in the risk estimates for methyl chloride presented in Tables V-1 to V-8 are: (i) the selection of the interspecific scaling factor (with an implicit assumption about pharmacokinetic differences), and (ii) the assumption that humans would be equal in susceptibility to male mice. Each of these assumptions leads to uncertainty of an order of magnitude or more, but the two assumptions are not independent. The choice of a dose-response model and the selection of adjustment factors for partial-lifetime exposure introduce uncertainties of smaller magnitude (probably factors of 2 to 4), except when the dose-response relationship is extrapolated below risk levels of 10^{-3}, where uncertainties increase rapidly. The estimates in Tables V-1 to V-8 are "conservative" in two respects: (i) the assumption that humans would be as

susceptible as the more susceptible sex of the more susceptible species; and (ii) the selection of dose-response models that give relatively high estimates of risk at risk levels below 10^{-3}. The risk assessments should be interpreted cautiously in the light of these uncertainties and biases.

APPENDIX A

AN EPIDEMIOLOGICAL STUDY OF WORKERS POTENTIALLY EXPOSED TO BROMINATED CHEMICALS

Tabershaw Occupational Medicine Associates (270) reported the results of an historical prospective study of workers employed by Velsicol Chemical Company (including Michigan Chemical Company, which merged with Velsicol in 1976). The cohort consisted of Velsicol workers who had been employed between 1935 and 1976 at four sites where potential exposure to brominated chemicals existed: manufacturing plants at St. Louis, Michigan; Manistee, Michigan; and El Dorado, Arkansas; and a research facility at Ann Arbor, Michigan. Demographic data and work histories were compiled from personnel records maintained at three company locations; work histories appear to have been incomplete for many workers, and dates of termination had to be estimated for 218 employees (5.17% of the cohort). Potential exposures to various brominated chemicals were inferred from the product-departments in which the workers had been employed. The cut-off date of the study was December 31, 1976. Vital status at the cut-off date was determined by local follow-up (2,368 persons), through Social Security Administration records (1,341 persons), or through state Motor Vehicle Bureaus (362 persons). Vital status could not be determined for 279 persons (6.6% of the cohort). Information on causes of death was obtained from death certificates, but no mention was made of attempts to verify this information.

Altogether 3.612 males and 602 females were included in the follow-up. Data on females were too meager for statistical analysis. Thirty-three males whose dates of birth were not known were excluded from the analysis, leaving a total of 3,579 males for study. All of these were "white" (Caucasian with a small minority of Spanish Americans). A majority of the workers was born between 1915 and 1934 (49.9%) and started work between 1940 and 1954 (62.8%). A majority of workers was employed for less than 1 year (53.6%); only 11.5% were employed for more than 10 years. The vital statitics of 94.5% of the cohort was known at the end of the study, and death certificates were obtained for 541 (93.6%) of the 578 workers known to have died.

Death rates for the cohort were compared with age-, sex-, and cause-specific mortality rates for the U.S. white male population for 5-year time periods between 1925 and 1975, and standard mortality ratios (SMRs) were calculated. The death rate from all causes was significantly less than that expected in a matched sample from the U.S. population (543 observed versus 614 expected, SMR = 88, $p<0.01$), indicating a "healthy worker effect." The SMR for all cancers was 102; death rates were not significantly elevated for cancers of any specific site or organ system, but the death rate for cancers of the digestive system was significantly lower than expected (18 observed versus 32.5 expected, SMR = 55, $p<0.05$). Among other causes of death, death rates from diseases of the circulatory

system and cirrhosis of the liver were significantly lower than expected, but the death rate from diabetes mellitus was significantly elevated (19 observed versus 8.7 expected, SMR = 220, $p<0.01$).

Breakdown of the mortality experience of the cohort by duration of employment and by length of follow-up showed no additional significant findings. Analysis of mortality data for workers potentially exposed to dibromochloropropane (DBCP) and tris-(2,3-dibromopropyl)phosphate (TRIS) showed no significant excess death rates from any cause, except for arteriosclerotic heart disease in DBCP-production workers. However, data for persons potentially exposed to other brominated organic and inorganic chemicals showed significant excess death rates from respiratory cancers (10 observed versus 4.4 expected, SMR = 228, $p<0.05$) and cancer of the testis (2 observed versus 0.17 expected, SMR = 1,198, $p<0.05$). Table A-1 summarizes the characteristics of the two workers who died from testicular cancer. "Based on the work histories, methyl bromide is the only common potential exposure, although the second individual worked later in the research laboratory with potential exposure to a multitude of chemicals." Both died at relatively young ages (27 and 33), but this was stated to be not unusual for testicular cancer victims. Both started work with methyl bromide at age 20, and they died 8 and 13 years later.

Although this study was conducted according to standard methodology, it suffers from a number of limitations. The

TABLE A-1

CHARACTERISTICS OF THE TWO DECEDENTS WITH CANCER OF THE TESTIS POTENTIALLY EXPOSED TO ORGANIC BROMIDE

Race	Date of Birth	Date of Hire	Date of Termination	Age at Death	Length of Employment	Work History	
White	03-24-47	04-13-56	11-15-64	27	3 yrs 5 mos	DDT	04-13-56 to 01-02-57
						Salt	02-04-57 to 07-17-57
						DDT	02-04-57 to 07-17-57
						Lig Cl_2	07-17-57 to 09-16-57
						CH_3Br_2*	09-16-57 to 01-20-58
						DDT	01-20-58 to 04-07-58
						MgO	04-07-58 to 09-07-59
White	11-06-35	04-18-56	02-03-67	33	10 yrs 3 mos	CH_3Br_2*	04-18-56 to 09-16-57
						$C_2H_5Br_2$**	09-16-57 to 02-03-67
						Research	09-16-57 to 02-03-67

*sic: presumably CH_3Br was meant

**sic: presumably C_2H_5Br was meant

SOURCE: Tabershaw Occupational Medicine Associates (250)

most important of these is the total lack of information on exposure. Apparently all workers at plants "where potential exposure to brominated compounds existed" were included in the study. The classification of workers by department was based on records that were clearly incomplete. Job titles apparently were not used and no industrial hygiene data were available. Thus, it is likely that many workers in the cohort had little or no exposure to brominated compounds. In addition, the high rate of job turnover meant that comparatively few workers were employed for more than 10 years. Although mortality data were tabulated by duration of employment and by length of follow-up, data were not presented for the critical sub-group of workers with both long potential exposure and long follow-up. The proportions of workers who were untraced or whose death certificates were not located were also larger than is desirable in studies of this type, and the failure to verify causes of death may have been a source of bias.

In these circumstances, the finding of two deaths from testicular cancer in workers potentially exposed to methyl bromide is no more than suggestive evidence for a casual association. The number of workers with potential exposure to methyl bromide was not stated, but was presumably much less than the 665 workers with potential exposure to "brominated organic chemicals." Thus, the SMR of 1,799 for testicular cancer in workers with potential exposure to brominated organic chemicals is probably much less than that would be calculated

for methyl bromide alone. However, the facts that only two unconfirmed cases were reported, that the latent periods were short, and that no specific information on exposure was available, makes this finding inconclusive.

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