Technical Report

No 32

Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data

May 1988

ISSN-0773-8072-32

MAY 1988

Technical Report

N° 32

METHYLENE CHLORIDE (DICHLOROMETHANE) : HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

Written of behalf of ECETOC by
T. GREEN
W.M. PROVAN
P.H. DUGARD
S.K. COOK

ICI Central Toxicology Laboratory, Alderley Park,
Macclesfield, Cheshire, SK10 4TJ, UK.

ISSN 07773-8072-32

METHYLENE CHLORIDE (DICHLOROMETHANE):

HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

ECETOC Technical Report No: 32

This document has been prepared and reviewed with all possible care by experts on behalf of ECETOC. It is provided solely for information. It is not to be taken as a warranty for which we take legal responsibility.

"This study is part of a major programme of scientific research being carried out at the Central Toxicology Laboratory of Imperial Chemical Industries PLC under the joint sponsorship of the member Companies of the CEFIC (Counseil Europeen des Federations de l'Industrie Chiminque) Methylene Chloride Sector Group with professional monitoring by ECETOC (European Chemical Industry Ecology and Toxicology Centre)".

METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

CONTENTS

CONTENTS - Continued

METHYLENE CHLORIDE (DICHLOROMETHANE):

HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

SUMMARY

Data obtained as part of the CEFIC/ECETOC research programme into the species differences in carcinogenicity of methylene chloride have been used in a physiologically based pharmacokinetic model to evaluate the carcinogenic risk to man from exposure to this chemical. The model has been based on evidence that the glutathione-S-transferase pathway is responsible for the tumours seen in exposed mice and is the cause of the species difference. Kinetic constants measured in vivo in mice, together with species-dependent physiological parameters, have been used to determine the target organ doses of glutathione-S-transferase metabolites. Metabolic constants measured in vitro have been used to scale from the in vivo constants measured in mice, to rats, hamsters and man. This procedure has been validated against in vivo rat data and against a range of human studies in the literature. The relationship between glutathione-S-transferase metabolites and tumours, established in mice, has been used to determine the risk associated with the internal dose of glutathione-S-transferase metabolites calculated by the model for man. The low risks calculated by this procedure indicate that man is adequately protected from the risk of methylene chloride induced cancer by the current hygiene standards which are based on the formation of carboxyhaemoglobin from this chemical.

1. INTRODUCTION

Methylene chloride (dichloromethane) is a volatile liquid, boiling point 40°C, used as an ingredient in paint stripping and aerosol preparations and as a solvent in a wide variety of industrial applications. A recent National Toxicology Programme (NTP 1986) life-time inhalation bioassay has shown an increased incidence of lung and liver tumours in $B_6C_3F_1$ mice after exposure to 2000 and 4000ppm methylene chloride. There were no corresponding increases in lung or liver tumours in F344 rats in the same study or in earlier inhalation studies in rats or hamsters exposed at similar dose levels (Burek et al, 1984). Two further studies using lower doses administered either in drinking water (Serota et al, 1986a, b), or by gavage in corn oil (Maltoni et al, 1986), also failed to cause an increase in lung and liver tumours in mice and rats.

It is possible to use this type of animal carcinogenicity data for quantitative assessment of risk to humans exposed to methylene chloride. Such an assessment should, first of all, take into account whether methylene chloride is a genotoxic carcinogen. From the literature and from studies in the present programme (see ECETOC 1986 for review) no convincing evidence has been found to conclude that methylene chloride is genotoxic in animals. Consequently it would be appropriate to derive a risk assessment using a safety margin applied to the no effect level in the long term animal experiments. However, methylene chloride is genotoxic in some prokaryotes (ECETOC, 1986) and to cover the possibility that an undetectable genotoxic action in the target organs is responsible for the tumours seen in the lungs and livers of mice, alternative forms of risk assessment are possible.

One such assessment was made by the United States Environmental Protection Agency (EPA, 1985) using data from the NTP life-time inhalation bioassay. In these calculations certain components can be recognised. These are; hazard assessment (review of toxicity data for quality and relevance to man); establishment of a dose response relationship in animals and its extrapolation to low doses; conversion of the dose response relationship from animals to man; comparison of human exposure with the dose response

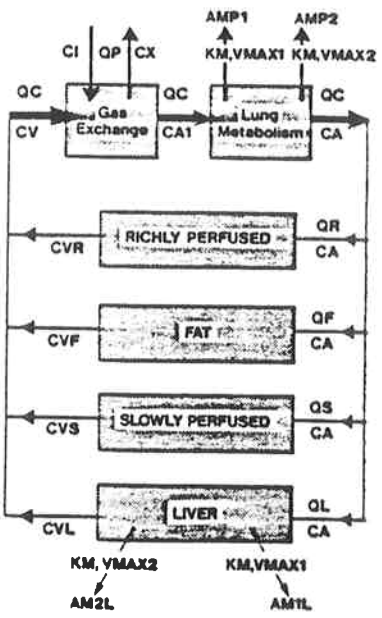
relationship to predict risk or doses associated with particular levels of risk. Standard assumptions are used by EPA to estimate the dose of methylene chloride during inhalational exposure of animals and the dose conversion to man is made on the basis of "equal response at equal doses per unit body surface area". The current EPA approach (1986) is considered to be extremely conservative, and designed to overestimate the risks to man.

Amongst the many uncertainties in the calculation of risk to man are that the relationship between the administered dose (external dose) in animal studies and the level of active metabolites in the sensitive tissues or cells is not known, nor can the differences between the animal and human dose-response relationships be identified. These uncertainties are reduced if the dose-response relationships in animals and man can be described in terms of tissue levels of key metabolites (internal dose) and if human exposures can be converted to the same parameters. This conversion is dependent upon a knowledge of the metabolism and pharmacokinetics of methylene chloride in the species of interest including man.

Detailed studies of the metabolism of methylene chloride have revealed two pathways with different enzyme/substrate affinities, metabolic saturation of one pathway, and both dose and species-dependent differences in the utilisation of the pathways (Green et al, 1986a,b; 1987a,b). The levels of methylene chloride and its major metabolites, carbon monoxide and carbon dioxide, have been measured in mice and rats both during and post-exposure over a range of dose levels from 100 to 4000ppm. The extent of metabolism by each pathway is also known in mice over the same range of dose levels (Green et al, 1987a). As a result of these studies evidence has accrued to suggest that the cancer seen in exposed mice is due to high rates of metabolism of methylene chloride by the glutathione-S-transferase pathway. The mechanism by which metabolites from this pathway induce cancer is unknown, methylene chloride being non-genotoxic both in vivo and in vitro in mammalian systems (ECETOC, 1986). The glutathione-S-transferase pathway has been shown to be relatively minor in rats in vivo (Green 1986a, 1987a) and in rats, hamsters and humans in vitro (Green 1986b, 1987b). At low dose levels methylene chloride is metabolised mainly by the cytochrome P-450 pathway in all species including mice (Green, 1987a).

It is clear from these studies that the dose and species-dependent metabolism of methylene chloride cannot be adequately described by the linear extrapolation from high to low dose and bodyweight or body surface area scaling (allometric scaling) used in conventional risk assessments (Howe 1983). Assessment of human risk from exposure to methylene chloride should take into account the metabolic and kinetic constants that describe the metabolism of methylene chloride by the glutathione-S-transferase pathway at different dose levels in each species. A mathematical model facilitating the calculation of an internal dose of metabolites of inhaled volatile chemicals has been described by Ramsey and Andersen (1984) and the use of such models in risk assessment has been discussed by Clewell and Andersen (1985). Recently the model was adapted for use with methylene chloride (Andersen et al, 1987). This report describes a development of that model using data obtained as part of the CEFIC-sponsored and ECETOC-monitored research into the species differences in carcinogenicity of methylene chloride (Green et al, 1986a,b; 1987a,b). The results permit a comparison between the standard approach used by EPA and the internal dose approach, which takes into account pharmacokinetic differences between species, in the evaluation of possible risks to humans exposed to methylene chloride.

2. MODEL STRUCTURE



Key: CI, inspired concentration, CX, expired concentration; QP, ventilation rate; QC, cardiac output; CA, arterial blood conc; CV, Venous blood conc. QF, QL etc blood flow to fat, liver compartments etc. Km, Vmax, biochemical constants.

The model used was basically that described by Andersen et al (1987) with only minor modifications. As before, the target tissues, the liver and lung, are treated as separate compartments whilst all other tissues are combined in groups displaying similar uptake and perfusion characteristics. Metabolism in the lung and liver compartments is now described by Michaelis-Menten constants for both the cytochrome P-450 and glutathione-S-transferase pathways. In its present form the model describes the behaviour of methylene chloride following inhalation exposure only.

3. MODEL PARAMETERS

3.1 Partition Coefficients

3.1.1. Coefficients used in the Model: Of the six partition coefficients used in the model, five relating to the partitioning of methylene chloride between blood and tissues have been measured in a number of species including man and rat (Fiserova-Bergerova, 1983). Hamster and mouse tissue partition coefficients were assumed to be the mean of the values measured in the other species. The other partition coefficient is a measure of the relative solubility of methylene chloride in blood and air. Details of the measurement of blood/air partition coefficients are given below. The values of all the partition coefficient used in the model are given in Table 1.

3.1.2 The Measurement of Blood/Air Partition Coefficients: Vial equilibration techniques (Sato and Nakajima 1979) have been used successfully to measure partition coefficients in a variety of species. In the present study consistent results were obtained for blood with this technique with the exception of the mouse blood:air partition coefficient. The value measured in mice using the vial equilibrium technique (Andersen et al, 1987) is approximately half of that measured with rat blood, which is somewhat untypical of the behaviour of other chlorinated solvents in the two species (Ward et al, 1988). Partition coefficients between mouse blood and air are normally found to equal or even exceed those found for rat blood and air. As a result of the apparent discrepancy with methylene

chloride a further technique was developed to measure blood:air partition coefficients. This involved a dynamic flow system in which blood could be exposed to any atmospheric concentration of methylene chloride. Full details of this technique are given in Appendix 1.

Blood:air partition coefficients obtained by this method for rat and human blood were similar to those obtained using the vial equilibration technique. Mouse blood however gave a value approximately double that measured with the previous method. This higher value was consistent over a wide range of atmospheric concentrations and in view of the known similarities between rat and mouse blood for other solvents, was considered to be a more appropriate value. The value used for hamster blood was that reported by Andersen et al (1987).

3.2 Physiological Parameters

3.2.1 Parameters used in the Model: The physiological parameters were taken from Andersen et al (1987) and Fiserova-Begerova and Hughes (1983) and are given in Tables 1 and 2. In the model described by Andersen the physiological parameters were assumed to be constants which were related between species or between organs by an allometric equation involving some exponent of the body weight. For example, cardiac output (QC) and ventilation rate (QP) were defined in this way and as such were independent of both dose and the activity of the animal. In the present model both QP and QC are treated as variables changing either with dose or work rate.

3.2.2 The Influence of Dose and Work Rate on QP and QC: It has become apparent that, during prolonged exposure to high concentrations of methylene chloride both QP and QC are variables with both dose and time. It is well established that during exposure of rodents to solvents including methylene chloride (Heppel et al, 1944; Thomas et al, 1972; Kjellestrand et al, 1985; Hext et al, 1986), that there is a complex relationship between dose and motor activity particularly in mice. At low doses or during the initial period of high doses, activity is increased whilst at high doses this increase in activity is quickly followed by a decrease for the remainder of the exposure period. This behaviour was apparent during a 6hr exposure of $B_6C_3F_1$ mice to the dose levels of

methylene chloride used in the NTP study (NTP, 1986; Hext et al, 1986). As a result of this change in activity, oxygen consumption and therefore ventilation rate and cardiac output also exhibit similar biphasic profiles.

Under the closed chamber conditions used by Andersen et al (1987) in which the methylene chloride atmosphere was allowed to decline, only the initial increase in QP and QC was modelled. This increase was expressed in the model as a doubling of the values of QP and QC for mice. The present model, which describes the metabolism of methylene chloride under steady state conditions, varies both QP and QC to take into account the initial increase at low dose levels and the more prolonged decrease seen under steady state conditions at high dose levels. The control values of QP and QC are derived using the allometric equation, QC or QP = 15 $(BW)^{0.74}$ litres/hr (Andersen et al, 1987). The increase induced at low exposure concentrations was modelled using 45 $(BW)^{0.74}$ litres/hr for QC and 135 $(BW)^{0.74}$ litres/hr for QP. At the highest exposures (4000ppm) under steady state conditions, the values of QP and QC are given by 7.5 $(BW)^{0.74}$ litres/hr.

Taking these changes into account the model over-estimates the blood levels of methylene chloride in the mouse (Green et al, 1986a) by a factor of 5 at the highest dose. In order to describe the experimental blood levels accurately at 4000ppm the proportion of the inhaled concentration of methylene chloride reaching the alveolus must be reduced by half. It can be assumed that a gradient exists at equilibrium at high doses between inspired concentration and that in the alveolus, probably due to a combination of reduced ventilation efficiency and the high rate of metabolism of methylene chloride found in the mouse. This phenomenon appears to be linked to ventilation rate and only takes effect at dose levels higher than 500ppm when ventilation rate is reduced. Although ventilation rate may be reduced in the rat and hamster the impact on blood levels is minor due to the lower metabolic rates in those species. All other physiological parameters, with the exception of animal body weights which are those used in the CEFIC/ECETOC studies, are as described by Fiserova-Bergerova and Hughes (1983) and are shown in Table 1. In predicting internal doses during the NTP study (NTP, 1986) a mouse body weight of 0.0325kg was used.

In man, at exposure levels of up to 500ppm methylene chloride, there is no effect on either blood circulation (QC) or respiration (QP) (Astrand et al, 1975). Consequently the standard allometric relationship ($15\ (BW)^{0.74}$ litres/hr) can be used to describe QP and QC in man in the absence of direct measurements. Although exposures of up to 500ppm methylene chloride do not change QP and QC, both values increase significantly with increasing work load (Astrand, 1983) and are therefore present as variables in the model describing human risk (Table 2). The work load asssociated with normal human occupational exposure is assumed to be 50 watts (50W).

3.3 Biochemical Constants

3.3.1 Metabolic Rate Constants used in the Model: In vitro values of Km and Vmax were calculated from a series of studies (Green et al, 1986b, 1987b) measuring the rate of metabolism of methylene chloride by the cytochrome P-450 and glutathione-S-transferase pathways in tissues from each of the four species (Tables 4 and 5). Their derivation is given in Appendix 2. In vivo values of Km and Vmax were obtained from the experimental mouse data (Green et al, 1986a, 1987a) by computer optimization (Tables 4 and 5). A comparison of the experimental data with that predicted by the model using these values is given in Table 3. The optimization procedure took into account the fact that carbon dioxide is derived from both metabolic pathways and that the amount from each pathway varies with dose (Green et al, 1987a). For the other species where the same depth of in vivo data is not available, in vivo values of Km and Vmax were obtained by using the experimental in vitro values to scale from the in vivo rate constants obtained in the mouse (Tables 4 and 5).

3.3.2 The Relationship Between In vivo and In vitro Rate Constants and their use in the Model: A marked difference is apparent between the Km values measured in vitro and those obtained from the mouse in vivo studies. This difference of two orders of magnitude is common to both pathways and is due to the difficulties in presenting a poorly soluble substrate like methylene chloride to an enzyme, when that enzyme is present in a dilute aqueous solution of liver homogenate at a temperature (37°C) close to the boiling point (40°C) of methylene chloride. This explains why the apparent Km's measured in vitro are considerably higher than those in vivo.

It is clear that the in vivo Km for the cytochrome P-450 pathway describes accurately the known saturation of this pathway at low dose levels, whereas the in vitro value would not have reached Vmax even at the 4000ppm dose level. A similar difference exists between the in vivo Km for the glutathione-S-transferase pathway and that measured in vitro (Tables 4 and 5). The highest in vivo Km values, 55mg/l and 0.39mg/l for the glutathione-S-transferase and cytochrome P-450 pathways respectively, are 2 within the range of available substrate concentrations measured in exposed rats and mice. In rats exposed to 4000ppm the equilibrium blood level of methylene chloride during exposure was 250mg/l and in mice 50mg/l (Green et al, 1986a). Consequently it is appropriate to treat both pathways as saturable pathways described by Michaelis-Menten kinetics. This change has been incorporated into the present model structure which now differs from that of Andersen et al (1987) where the glutathione-S-transferase pathway was described by a first-order rate constant Kf.

In the derivation of the values given in Appendix 2 and Table 5 for the glutathione-S-transferase pathway in hamsters and in man, certain assumptions were made. Km and Vmax were not available for this pathway in these species either in vivo or in vitro. Appropriate studies do not exist in vivo and the in vitro metabolic rate data is available only at a single substrate concentration (35mM). The Km value in the hamster and in man has been assumed to be the same as that in the rat (21mM), since metabolic rates in these species more closely resemble those in the rat than the mouse. If this assumption is made it follows that the rate measurements at a 35mM substrate concentration are approaching Vmax. The lower Km (21mM), as opposed to the higher value measured in the mouse (86mM), will give the highest metabolic rates at the lower dose levels found in man and hence can be considered to be the more conservative assumption. Any further error in the choice of Km for man is unlikely to greatly affect the outcome of the model. At the low concentrations of methylene chloride associated with human exposure the metabolic rate can be assumed to be first order and related linearly to the concentration of methylene chloride in the target organs.

3.3.3 Metabolic Rate Constants used in the Lung Compartment

Significantly less data are available describing the metabolism of methylene chloride by the two pathways in the lungs of the various species. In vitro rate data is available at a single substrate concentration for the cytochrome P-450 pathway in rat, mouse and hamster lung and for the glutathione-S-transferase pathway in mouse lung. These studies have shown that cytochrome P-450 metabolism in the lung, expressed as activity per mg protein, is comparable to that in the liver for the three animal species (Green et al, 1986b). In the absence of human lung data it has been assumed that the same relationship between the two organs holds and that cytochrome 2P-450 metabolism of methylene chloride in human lung is similar to that in human liver.

Glutathione-S-transferase activity in the mouse lung was found to be one-tenth of that in mouse liver when assayed with both methylene chloride and chlorodinitrobenzene (Green et al, 1986b). Although data were not available describing the metabolism of methylene chloride by lung tissue from the other species, the rates in these species with chlorodinitrobenzene were also one-tenth of those in the liver. Consequently it has been assumed that the metabolism of methylene chloride in lung tissue from rats, hamsters and man occurs at a rate one-tenth of that in the liver of these species. This assumption is consistent with the fact that the assay procedures were unable to detect activity in the lungs of the other species.

4. MODEL VALIDATION

4.1 In Vitro Scaling Between Species

Evidence has been obtained to suggest that the lung and liver cancer seen in mice exposed to methylene chloride is due to high rates of metabolism by the glutathione-S-transferase pathway in this species. Significantly lower rates in rats and hamsters are consistent with the lack of carcinogenicity in these species, and the lowest rates, found in human tissues, suggest that the human risk is minimal. This information forms the basis of a risk

assessment model in which risk is based on the degree of glutathione-S-transferase metabolism *in vivo* in mice and man. Extensive *in vivo* data now exists in the mouse to determine this. However, in man this pathway has never been measured *in vivo* nor can this be done without the use of unacceptably high doses of radiolabelled methylene chloride. The only measure of this pathway in man is *in vitro* studies using human tissues. These studies in comparison with identical studies using mouse, rat and hamster tissues *in vitro* provide a means of scaling from the *in vivo* data available in mice.

In order to extrapolate from the mouse to man using *in vitro* data it is important to demonstrate that metabolic rates measured in this way are an accurate representation of the metabolism of methylene chloride *in vivo* in the various species. The accuracy of this type of scaling can be established, because, in addition to the mouse, *in vivo* data is available in the rat describing both blood levels of methylene chloride and its metabolism to carbon monoxide and carbon dioxide over a wide range of dose levels. Consequently metabolic rate constants obtained for the rat by *in vitro* extrapolation can be validated against this *in vivo* data base.

Values of Km and Vmax for each pathway in the rat, were used in the model to predict the blood levels of methylene chloride and the levels of carbon monoxide and carbon dioxide in exhaled air during exposures to 500 and 4000ppm. The ability of *in vitro* scaling to predict the *in vivo* behaviour of methylene chloride in the rat is shown in Table 6. As can be seen from the measured and predicted values shown in Table 6, this technique accurately describes both the uptake and metabolism of methylene chloride in the rat over this range of dose levels. It can therefore be assumed that hamster and human metabolic constants obtained by the same procedure also describe the behaviour of methylene chloride in these species *in vivo*.

4.2 Studies in Man

Although in laboratory animals we frequently have to rely on blood level measurements to estimate the internal dose of methylene chloride, in man other data is often available to measure uptake. The difference between the inhaled and exhaled breath concentrations of exposed volunteers as a

product of their respiration rate provides the pulmonary uptake or internal dose of methylene chloride. Fortunately there are sufficient controlled exposure experiments with methylene chloride in man to allow the internal dose predictions made by the human model to be verified (Astrand et al, 1975; Engstrom and Bjurstrom, 1977; DiVincenzo and Kaplan, 1981).

However, although uptake can readily be determined, in vivo data is not available describing the metabolism of methylene chloride by the glutathione-S-transferase pathway. Information is available describing pulmonary uptake, blood and fat concentrations of methylene chloride, pulmonary clearance of carbon monoxide during exposure and carbon monoxide and methylene chloride following exposure. Furthermore, much of this data is available for volunteers either at rest or during varying degrees of exercise. Consequently if the human model, which is based on in vitro scaling, can successfully predict this large variety of results at different dose levels and under different physiological conditions, it is highly probable that the model will also predict glutathione-S-transferase metabolism correctly.

The available human in vivo studies (Astrand et al 1975, Engstrom and Bjurstrom 1977, DiVincenzo and Kaplan 1981) allow validation of the model under conditions where either metabolism or tissue distribution are the major factors in determining uptake. In addition we can validate the effects of exercise (work-load), not only on total uptake but also as it affects metabolism and tissue uptake.

4.2.1 Uptake of Methylene Chloride: DiVincenzo and Kaplan (1981) reported the pulmonary uptake, or internal dose of methylene chloride, at four dose levels from 50-200ppm. These results together with those predicted by the human model are shown in Table 7. Astrand et al (1975) also reported the pulmonary uptake of methylene chloride at doses up to 500ppm during different degrees of exercise. A comparison of predicted and experimental results is shown in Table 8. Engstrom and Bjurstrom (1977) used an even higher dose level of 750ppm, exposing volunteers performing work at 50W for one hour. The results of this comparison are shown in Table 7.

Each of the comparisons between the model and experimental data show the model to represent accurately the uptake of methylene chloride in humans under a variety of conditions. Uptake approximately doubles between rest and a work-rate of 50W and thereafter remains constant with increasing work-rate (Astrand et al, 1975). At the higher work-rates (100 and 150W) cardiac distribution is a critical factor in the uptake, and may vary significantly between individuals. In the example shown in Table 8 average values of cardiac distribution were used since individual measurements were not available. These measurements would improve the accuracy of the model at high work-rates.

4.2.2 Metabolism by the Cytochrome P-450 Pathway: The relative contributions of tissue uptake and metabolism to total uptake vary according to dose and exercise. The low dose studies of DiVincenzo and Kaplan (1981) provide important information about the internal dose associated with metabolism. At rest and at the low doses used in these studies metabolic uptake accounts for a large proportion of total uptake. Figure 1a shows a model prediction of tissue, metabolic and total uptake of methylene chloride in individuals at rest in an atmosphere of 50ppm.

In vitro and in vivo studies in animals (Green et al, 1986b, 1987a,b) have shown that metabolism at low dose levels is primarily by cytochrome P-450 and hence at the dose levels used by DiVincenzo and Kaplan (1981) metabolic uptake becomes a good approximation to the rate of metabolism by cytochrome P-450. Although, from Figure 1a, uptake is clearly dependent upon metabolic rate at dose levels of <100ppm, metabolism itself is substrate limited and hence ventilation rate and cardiac output are the major factors influencing uptake. As the dose increases metabolism is no longer substrate limited and hence the sensitivity of uptake towards changes in Vmax increases. Figure 1b illustrates the sensitivity of uptake at a 200ppm dose level to changes in Vmax. The total uptake reported by DiVincenzo and Kaplan (1981) at 200ppm, shown in Table 7 together with the model predictions, comprises largely of metabolic uptake and is entirely consistent with the metabolic rate for the cytochrome P-450 pathway used in the model.

Similar studies, using higher dose levels, reported by Astrand et al (1975), which measured uptake as a function of dose and exercise also help to validate the metabolic rate used in the model. In these studies uptake was measured over a shorter time course (0-2hr) and at higher work loads (up to 150W) than those studies of DiVincenzo and Kaplan (1981). They are therefore generally more sensitive to the factors controlling tissue rather than metabolic uptake. However, at the lower work loads studied by Astrand et al (1975) metabolic uptake is still a significant proportion of total uptake, approximately 50% (Figure 2a). The total uptake is shown in Table 8 together with the model predictions. Again this experimental data is entirely consistent with our metabolic rate estimated from the in vitro studies and scaling from the mouse in vivo data.

4.2.3 Validation Against Other Studies: This section deals with the validation of the model against other human experimental data, namely, elimination of carbon monoxide and blood levels of methylene chloride. Engstrom and Bjurstrom (1977) measured uptake of methylene chloride in human volunteers at a dose (750ppm:1 hour) at which 85% of the total uptake is represented by tissue uptake and is distributed at the end of exposure as shown in Figure 3.

The amount of carbon monoxide produced during and following exposure of volunteers to methylene chloride (50-200ppm) was reported by DiVincenzo and Kaplan (1981). Assuming that man, like the mouse, expires only part of the total cytochrome P-450 metabolism as carbon monoxide (Green et al, 1987a), comparison can be made between the model predicted carbon monoxide levels and those from the human volunteers (Table 7).

Finally blood levels of methylene chloride are readily available from studies at several different dose levels and under differing physiological conditions. An example from the work of Astrand et al (1975) illustrating a complex protocol of two dose levels, breaks during exposure and different work rates, is shown in Figure 2b together with the model predictions.

It is clear from the above examples that the model is able to predict the behaviour of methylene chloride in humans under a variety of conditions. These have included a range of dose levels, including doses where either

metabolic or tissue uptake control total uptake and a variety of physiological conditions which affect uptake and the distribution of cardiac output. Uptake, blood levels, and the elimination of carbon monoxide and methylene chloride in expired air, have all been accurately predicted by the model.

5. INTERNAL DOSE CALCULATIONS

5.1 Experimental Animals

Using the physiological parameters the model has been used to convert external dose to an internal dose of methylene chloride in the liver and lungs of each species exposed under the conditions of the NTP lifetime inhalation bioassay. Use of the pharmacokinetic constants (Tables 4 and 5) enables the areas under the tissue concentration/time curves for the metabolites from each pathway (AM1 and AM2) in liver and lung tissue from each species of interest to be calculated. The latter terms divided by the volumes of the tissues provided the internal dose for each pathway in each target tissue (Andersen et al, 1987).

$$\text{Internal dose 1}_{(\text{tissue})} = \frac{\text{AM1}_{(\text{tissue})}}{V_{(\text{tissue})}}$$

$$\text{Internal dose 2}_{(\text{tissue})} = \frac{\text{AM2}_{(\text{tissue})}}{V_{(\text{tissue})}}$$

The relationship between external dose of methylene chloride and the internal dose of metabolites from each pathway is shown for each animal species for a period of 24hrs (Figures 4,5) during which the animals are exposed for 6 hours at concentrations from 100 to 4000ppm. From these plots the dose-dependence of the two pathways and the marked species differences in the utilisation of the two pathways is clear.

Table 9 shows a comparison of the 24 hour internal doses of cytochrome P-450 and glutathione-S-transferase metabolites in the lungs and livers of

rats, mice and hamsters exposed to methylene chloride, with the tumour incidences in the bioassays (NTP 1986; Burek et al, 1984). As observed previously from the experimental studies (Green et al, 1986a,b, 1987a,b), a clear relationship exists between glutathione-S-transferase metabolites in the three species and the observed tumour incidences in either the liver or the lung. At the same time no such relationship exists for the cytochrome P-450 pathway. The internal dose calculations shown are based on data obtained from experiments comprising single exposures and throughout the subsequent risk calculations it is assumed that the same daily dose applies throughout the lifetime studies.

5.2 Man

For the purpose of defining human risk, occupational exposure of a 70kg worker has been defined as 8 hours per day of work at 50W, followed by 16 hours at rest, 220 days per year for 35 years of a 70 year lifetime. The internal doses of glutathione-S-transferase metabolites for humans, calculated for a 24 hour period during which man is exposed to external doses of between 10 and 500ppm for the first 8 hours, are shown in Figure 6.

The computer programmes used to calculate the internal doses are available from the authors on request.

6. CALCULATION OF HUMAN RISK

6.1 Dose-response Modelling using Internal Dose

Three mathematical models were considered for the dose-response extrapolation; the multistage, "one-hit" and Weibull models. It is not possible when considering internal doses to combine liver and lung tumour incidences. Each tissue must be regarded as displaying an independant dose-response relationship. The "one-hit" model provided a poor fit to the experimental data for both lung and liver and in the absence of any biological justification for its use, the results are not displayed here.

The multistage model was fitted using the computer programme GLOBAL 82 (Crump and Associates Inc) and the Weibull model using the Weibudda programme (Crump and Associates Inc). The tumour incidences used were those in the female mouse in the NTP inhalation bioassay and were expressed as "any animal with adenoma and/or carcinoma". The details are as follows.

	Experimental Dose Level (ppm)		
	0	2000	4000
Hepatocellular adenoma or carcinoma	3/50	16/48	40/48
Alveolar/bronchiolar adenoma or carcinoma	3/50	30/48	41/48

The general mathematical expression for the multistage dose response model has the form:

$$Pd = 1 - \exp(-q_0 - q_1 d - q_2 d^2 - q_3 d^3 .....)$$

Where "Pd" is the probability of a cancer developing (background and compound-induced) at a dose "d", "q" is related to the probability of a cancer developing at zero dose [$P_0 = 1-\exp(-q_0)$] and "q_0, q_1 etc" are constants.

The extra risk "Rd" contributed by a dose "d" is

$$Rd = 1 - \exp(-q_1 d - q_2 d^2 - q_3 d^3 .....)$$

At low doses this is approximately:

$$Rd = q_1 d + q_2 d^2 + q_3 d^3 .....)$$

The multistage model has reasonable flexibility for fitting experimental data since it allows the possibility of linear, dose squared, dose cubed or even higher power terms to be used. However, the "two dose level and control" design of the NTP mouse study limits the multistage equation to linear and dose squared terms only.

The Weibull model has the form:

$$Pd = 1 - \exp(-\alpha - \beta d^{\gamma})$$

and at low doses the relationship between extra risk and dose is approximately:

$$Rd = \beta d^{\gamma}$$

Thus the Weibull model fits risk to "γ", some power (not necessarily a whole number) of dose and a proportionality coefficient "ß". Both the Weibull and multistage models are "non-threshold" in character and thus will indicate that a finite risk of cancer being induced exists for even the smallest dose.

The internal doses of glutathione-S-transferase metabolites, are given in Table 9. Figures 7 and 8 show the pattern of observed tumour incidences in mice relative to internal dose together with the curves fitted by application of the multistage and Weibull models. The Weibull model fits the observed dose response exactly for both liver and lung, however, it should be noted that the Weibull model will because its very nature, fit any three points in the pattern shown. The multistage fit for each organ does not include a term that is linear in dose because Global 82 is constrained to reject a negative linear term which would arise for this dose response pattern ie. a fall in tumour incidence with rising dose is considered biologically implausible. There is a further constraint affecting the multistage treatment, which is the "two-dose levels plus control" design of the NTP bioassay. This limits the multistage equation to linear and dose squared terms. Had more dose levels been employed, terms in dose cubed or higher powers might have improved the multistage fit of the data. The maximum likelihood estimates (best estimate) parameters, ie. derived from the fitted line, which describe the dose-response relationship are:-

Liver:- Multistage $\quad Risk = q_2 \times dose^2$

where $q2 = 4.8623 \times 10\text{-}8$

Weibull $\quad Risk = \beta \times dose^{\gamma}$

where $\beta = 5.3525 \times 10^{-22}$ and $\gamma = 5.838$

Lung:- Multistage $\quad Risk = q_2 \times dose^2$

where $q2 = 5.9474 \times 10\text{-}6$

Weibull $\quad Risk = \beta \times dose^{\gamma}$

where $\beta = 6.5444 \times 10^{-8}$ and $\gamma = 2.732$

The parameters shown relate to the lines of best fit to the experimental data which are also known as the curves of maximum likelihood. Another approach used by some is to consider the upper 95% confidence limit of risk for given doses rather than the maximum likelihood estimates of risk (parameters for this calculation are given in Appendix 3). Used in this way, the confidence limit represents an arbitrary safety factor which is the product of the curve fitting process. The confidence limit is based on the precision of the parameters in the model and does not reflect the variability between models or the uncertainty in the experimental data. The two bioassays in which methylene choride did not induce tumours in mice (Serota et al, 1986b; Maltoni et al, 1986) support the predicted steep dose response relationships shown in Figures 7 and 8. For these patterns of dose response relationships the predictions of risk based on confidence limits and best estimates show a wide divergence with the factor of difference increasing as the dose is reduced.

6.2 Estimates of Human Risk using Dose Response Models based on Internal Dose

Calculations of human risk using dose response relationships described in 6.1 assume that man will respond with the same tumour incidence as the female mouse at the same internal dose of glutathione-S-transferase metabolites. The human exposure considered is that of a 70kg human for 8 hours each working day, 220 days per year for each of 35 years of a 70 year lifetime. Starting from a given atmosphere of methylene chloride, the internal dose to lung and liver in terms of glutathione-S-transferase metabolites is calculated for a 24 hour period using the pharmacokinetic model.

This initial internal dose is then adjusted, using the exposure conditions appropriate, to a lifetime average daily dose (LADD) over a 70 year lifespan.

$$\text{LADD} = \text{working day internal dose} \times \frac{35}{70} \times \frac{220}{365}$$

A further correction is applied to take into account that mice were exposed 5 days per week and the LADD is then used to calculate risk using the model parameters and relationships described in 6.1 above. The results of these calculations, displayed in Figures 9 and 10 and Table 10, show estimates of risk for working life exposures to atmospheres of methylene chloride.

The estimates of risk shown in Table 10 are the lifetime risk for a compound induced cancer. Thus a risk of 1×10^{-6} is that, for one million people exposed to the associated atmosphere throughout a working life, one person will develop a compound-related cancer at some stage in their lifetime. Although the estimates of risk vary between liver and lung or the combination of both organs, and from dose-response model to dose-response model, a working lifetime exposure to 500ppm is, in all cases, associated with a risk of less than 1×10^{-4}.

7. DISCUSSION

7.1 The Experimental Animal Data used in the Model:

In order to use species-dependent physiology and pharmacokinetics in risk assessment it is essential to demonstrate the power of this type of modelling to describe firstly, the behaviour of the chemical at different doses in the animal species where cancer is found, secondly to extrapolate to other animals where cancer data and possibly pharmacokinetic data may be available, and thirdly and most importantly, to man where very little if any experimental data may be available. Consequently considerable attention has been paid in this paper to demonstrating the ability of this

model to predict the kinetic behaviour and known carcinogenicity of methylene chloride in mice, rats and hamsters, and the ability of the model to fit a wide range of human data.

For the current model a very extensive experimental data base exists in the mouse describing the pharmacokinetic behaviour of methylene chloride over a range of dose levels from 100 to 4000ppm. Data also exists in rats *in vivo* and in mice, rats, hamsters and man *in vitro* (Green *et al*, 1986a,b, 1987a,b). In fact the model is based on the findings of these studies which have provided evidence for the role of the glutathione-S-transferase pathway in the species differences in carcinogenicity between mice and rats and hamsters. The *in vivo* and *in vitro* data available in both mice and rats have been used to show that *in vitro* scaling can be used to extrapolate across species to man, where the metabolic rate constants derived from scaling have been shown to describe experimental studies in man. The amount of carbon dioxide eliminated from each pathway at different dose levels has been determined in the mouse using stable isotopes (Green *et al*, 1987a) and this information has been used quantitatively in the model. The assumption is made that the isotope effects seen relate only to the rate-determining glutathione-S-transferase and cytochrome P-450 steps and that isotope effects in subsequent metabolic steps have no effects on the overall rates measured *in vivo*. Similarly the presence of deuterium in the intermediate formyl chloride is assumed not to effect either the chemical or enzymic reactions of this metabolite since loss of chlorine would be rate determining in either case. Any error in the assumption that isotope effects do not occur in subsequent metabolic steps would be conservative, tending to over estimate metabolism by the glutathione-S-transferase pathway in the model. However there is no reason to believe on either practical or theoretical grounds that the isotope effects measured do not accurately define the source of carbon dioxide and consequently they have been used in the model as reported.

7.2 The Lung Compartment in the Model

The description of the lung in the model may be less than ideal and as such is an area for future development. In its present form the model contains a gas exchange compartment with blood flowing from this compartment to an

additional lung compartment where metabolism takes place. As the cells lining the airways, including the Clara cells, are directly in contact with an atmosphere of methylene chloride it seems likely that diffusion of a lipophilic chemical such as methylene chloride occurs directly into these cells without passing through the gas exchange compartment and into the blood. Thus the dose to these cells may be significantly higher than that defined by the parameters of gas exchange and blood flow. The ability of cells lining the airways to remove methylene chloride from the atmosphere may, in part, result in alveolar concentrations below those of inspired air. This appears to happen because the blood levels in the mouse can only be adequately described in the model if a gradient is assumed to exist in the airways resulting in the alveolar concentration being approximately one-half of the inspired concentration at the highest dose level. This phenomenon could equally be due to the changes in breathing rate believed to occur at high dose levels. A reduction in breathing rate or possibly increased upper airways breathing could result in methylene chloride being cleared from the alveolus by the blood faster than it is replaced.

Methylene chloride has been shown to be specifically cytotoxic to mouse lung Clara cells (Hext et al, 1986). Previous studies have also shown a change in the utilisation of the two metabolic pathways in Clara cells exposed to methylene chloride (Green et al, 1987c). Exposure results in a loss of cytochrome P-450 metabolism without any effect on the glutathione-S-transferase pathway suggesting an increase in carcinogenic risk to these cells. These studies provided circumstantial evidence that the Clara cell is the cell of origin of the tumours seen in the lungs of exposed mice.

Although the cytochrome P-450 enzymes are lost completely from damaged Clara cells, only 50% of the cytochrome P-450 activity is lost from whole lung homogenates (Green et al, 1987c). Identical results have been reported with 1,1-dichloroethylene which also damages the Clara cell in the mouse lung (Krijgsheld et al, 1983). From these studies it can be concluded that 50% of the total pulmonary cytochrome P-450 is located in the Clara cells which themselves account for approximately 5% of the total cell types in the mouse lung. The metabolic rate constants used in the model for the lung compartment are based on studies using homogenates of whole lung tissues and consequently do not represent the level of activity found in the Clara cells. If, as may be assumed, the distribution of the

glutathione-S-transferase enzymes follows that of cytochrome P-450, the activity of the glutathione-S-transferases in the Clara cells will be ten-fold higher than that in the whole lung. If the loss of cytochrome P-450 and the higher rates of glutathione-S-transferase metabolism in Clara cells are incorporated into the model, the internal dose to these cells is comparable to that of the liver (Table 9). In this calculation the Clara cells have been defined as a separate compartment in the model from the main lung compartment. The volume of the Clara cell compartment is set at one-twentieth of that of the lung. Cytochrome P-450 activity is set at zero and the glutathione-S-transferase activity is determined by the ratio of the specific activity in the Clara cells, which are assumed to contain 50% of the total pulmonary glutathione-S-transferase activity, to that in the liver.

The change in the utilisation of the two pathways and the differential activity in the Clara cells have not been used in the risk calculations for the lung at the present time. The treatment and exact measurement of changing metabolic rates in specific cell types is an area for further development beyond the scope of the present paper. Although not included at the present time it can be concluded that the use of this type of detail in the model will increase the margin of safety between mice and man since the loss of cytochrome P-450 appears to be specific to mice. Additionally the mouse lung contains significantly more Clara cells than human lung (Smith et al, 1979) thus reducing the risk even further. As a result the model in its present form is likely to overestimate, rather than underestimate, the risk to human lung.

7.3 Model Validation

As with most industrial chemicals shown to be animal carcinogens the number of human studies describing the behaviour and fate of methylene chloride is limited. Ideal studies describing the metabolism of methylene chloride by the glutathione-S-transferase pathway do not exist in man but there are a surprising number of complex experimental studies available (Astrand et al, 1975; Engstrom and Bjurstrom, 1977; DiVincenzo and Kaplan, 1981). These studies include details of the uptake of methylene chloride obtained from

measurements of inspired and expired air, blood levels, and details of the metabolism of methylene chloride to carbon monoxide. They also include other key information such as the effects of dose and work rate on uptake and tissue distribution. This data is important because there is a complex relationship in man between dose, work, uptake, metabolism and tissue distribution. For this reason validation of the model against a single blood level is inappropriate since the factors determining the blood level may be completely different at a higher or lower dose or different work rate. Fortunately a range of data is available and it is possible to validate all aspects of the models prediction of the behaviour of methylene chloride with the exception of glutathione-S-transferase metabolism. To accurately predict uptake, blood levels and metabolism to carbon monoxide implies that the single remaining parameter, that of glutathione-S-transferase metabolism, must also be predicted correctly by the model.

The ability to obtain this type of agreement between model predictions and experimental data for man and the correlation between dose and cancer in animals allows the human risk from exposure to methylene chloride to be predicted with some confidence. The accuracy of the predictions based on the liver will be more accurate than those based on the lung which is less well defined in the model for the reasons given earlier. In some cases the unknown variables in the definition of the lung compartment are constant between species and although they affect the absolute values of the internal dose in each species, they do not affect the relative risk between species. Other factors such as the variable numbers of Clara cells between species or the metabolic changes occurring only in the mouse lung would further reduce the risk to man. The model will therefore in its present form almost certainly overestimate the risk to human lung from exposure to methylene chloride.

7.4 Risk Assessment

The model is now able to describe the behaviour of methylene chloride in a variety of species, to take into account the fact that carbon dioxide comes from both pathways and to understand the relative risk to the lung and liver. Prediction of cancer risk by this model from one species to another

must of necessity assume that a given internal dose of critical metabolite(s) per unit of a target organ is associated with the same degree of risk in each species of interest. There is little point using modelling to predict risk unless this assumption is reasonably accurate. Clearly there will be instances when different species do respond differently to the same internal dose. In these cases either a more complex risk assessment is required taking into account those factors which cause the difference in response, or alternatively, a simple generic assessment may be appropriate when the species differences are clear cut and well understood. Chemicals which are rodent carcinogens as a result of peroxisome proliferation or hyaline droplet formation appear to fall into the latter category. However, in the case of methylene chloride the internal dose calculations shown in Table 9 provide a reasonable correlation between dose via the glutathione-S-transferase pathway and tumour incidence in mice. The calculations also indicate much lower doses in rats and hamsters via this pathway which is consistent with the absence of a carcinogenic response. The compatibility of the tumour incidence data with computed internal dose increases the level of confidence for the use of this type of model for the prediction of internal dose and risk in man. Nevertheless, it should be recognised that this type of modelling always contains a number of assumptions which cannot be tested directly, but only by validation with acute responses.

The patterns of the dose relationships for tumour incidence versus internal dose in the liver especially, but also the lung (Figs 7 and 8), are suggestive of a threshold i.e. the existence of a dose of glutathione-S-transferase metabolites which would not induce tumours. The presence of a threshold is compatible with the apparent non-genotoxic character of methylene chloride (ECETOC, 1986). Neither the Weibull nor the multistage models used here to fit the dose response data is capable of providing estimates of threshold doses but the Weibull, with its ability to generate a relationship in a power of dose geater than dose squared, comes closer to a threshold pattern. Thus, in this particular case, the Weibull model appears to fit the data better than the multistage treatment and to be more relevant to the probable mechanism of methylene chloride carcinogenicity. Overall, there are two reasons to expect the absence of detectable levels of tumour induction observed in the drinking water study and gavage studies

of Maltoni et al (1986) and Serota et al (1986b). The first is that methylene chloride is metabolised predominantly by cytochrome P450 at these dose levels and hence the levels of glutathione-S-transferase metabolites will be very low; the second is that these very low levels of glutathione-S-transferase metabolites, because of the steep dose response relationship, would generate only extremely small tumour incidences or even be below a threshold level for tumour induction.

The risks to both lung and liver, and the combined risks to both organs have been determined over a range of occupational exposures from 10-500ppm (Table 10). At the lower concentrations, more appropriate to current occupational exposure levels, the risk were as follows:

Exposure (ppm)	Risk, Lung + Liver (MLE)	
	Weibull	Multistage
10	3.554×10^{-12}	6.519×10^{-9}
50	5.650×10^{-10}	2.720×10^{-7}
100	4.959×10^{-9}	1.504×10^{-6}

Alternatively risks of 1×10^{-4} and 1×10^{-6} are frequently considered to be conservative levels of acceptable risk for occupational and general population exposures respectively. The exposures corresponding to these levels of risk are as follows:-

Risk (MLE) Liver + Lung	Exposure (ppm)	
	Weibull	Multistage
1×10^{-4}	>5000	1390
1×10^{-6}	1220	80

These results can be compared with those for a multistage treatment (linear term is accepted by Global 82) for combined lung and liver tumour incidence versus "external" methylene chloride dose when no pharmacokinetic modelling is applied. For this treatment, an occupational exposure of approx 0.06ppm is predicted to be equivalent to a risk of 1×10^{-4} and, assuming the same

pattern of exposure, a risk of 1×10^{-6} is equivalent to an atmosphere of 0.0006ppm. The very large factors of difference between these "external" dose estimates and those based on "internal" dose, which widen as the level of risk is reduced, arise from two sources. The first is that the metabolism information and pharmacokinetic modelling indicate that, for a given external atmosphere of methylene chloride, man generates a very much lower internal dose of active metabolites than the mouse. This is, of course taken into account in the treatment displayed in this report. The second is that the dose response relationship between tumour incidence and external dose is not as steep as that based in internal dose with consequent divergence as doses/risks are reduced.

It is clear from the values calculated using the internal dose that the exposures associated with a risk of 1×10^{-4} are several orders of magnitude higher than the hygiene standards currently operating in the workplace. Equally the likely exposures experienced by the general population either in ambient air, or through the use of methylene chloride in aerosols or food extraction, are also significantly lower than the levels associated with a risk of 1×10^{-6}. Consequently the short term effects of exposure to methylene chloride, rather than the long term risk of cancer, would seem to be the more appropriate basis for the determination of acceptable exposure levels. Current occupational limits, based on the metabolism of methylene chloride to carbon monoxide and the formation of carboxyhaemoglobin, appear to provide an adequate margin of safety.

REFERENCES

- Andersen, M.E., Clewell, H.J., Gargas, M.L., Smith, F.A. and Reitz, R.H.(1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87, 185-205.
- Astrand, I., Ovrum, P. and Carlsson, A.(1975). Exposure to methylene chloride I. Its concentrations in alveolar air and blood during rest and exercise and its metabolism. Scand J Work Environ Health 1, 78-94
- Astrand, I.(1983). Effect of physical exercise on uptake, distribution and elimination of vapors in man In:- Modelling of inhalation exposure to vapours: Uptake, distribution and elimination (V. Fiserova-Bergerova, ed), Volume II, pp108-130. CRC Press, Florida.
- Burek, J.D., Nitschke, K.D., Bell, T.J., Wackerle, D.L., Childs, R.C., Beyer, J.E., Dittenber, D.A., Rampy, L.W. and McKenna, M.J.(1984). Methylene chloride: A two year inhalation toxicity and oncogenicity study in rats and hamsters Fundam Appl Toxicol 4, 30-47.
- Clewell, H.J. and Andersen, M.E.(1985). Risk assessment extrapolations and physiological modelling. In:- Advances in Health Risk Assessment for Systemic Toxicants and Chemical Mixtures. Princeton Scientific Publishers, Princeton, NJ.
- DiVincenzo, G.D. and Kaplan, C.J.(1981). Uptake, metabolism and elimination of methylene chloride vapor by humans. Toxicol Appl Pharmacol 59, 130- 140.
- ECETOC.(1986). The assessment of carcinogenic hazard for human beings exposed to methylene chloride. Technical Report No: 26.
- Engstrom, J. and Bjurstrom, R.(1977). Exposure to methylene chloride. Content in subcutaneous adipose tissue. Scand J Work, Environ Health 3, 215-224.
- EPA.(1985). Addendum to Health Assessment Document for Dichloromethane (Methylene Chloride). EPA-600/8-82-004FF.
- EPA.(1986). Guidelines for carcinogen risk assessment. Federal Register 51, No: 185, September 24th 198
- Fiserova-Bergerova, V.(1983). Gases and their solubility: A review of fundamentals. In:- Modelling of inhalation to vapors and gases: Uptake, distribution and elimination (V. Fiserova-Bergerova, ed) Volume I, pp4-28. CRC Press Florida.
- Fiserova-Bergerova, V. and Hughes, H.C.(1983). Species differences in bioavailability of inhaled vapors and gases. In:- Modelling of inhalation exposure to vapors: Uptake, distribution and elimination (V. Fiserova-Bergerova, ed). Volume II pp98-106. CRC Press, Florida.
- Green, T., Provan, W.M., Nash, J.A. and Gowans, N.(1986[a]). Methylene chloride (dichloromethane): In vivo inhalation pharmacokinetics and metabolism in F344 rats and $B_6C_3F_1$ mice. Imperial Chemical Industries PLC. Central Toxicology Laboratory Report No: CTL/R/880.
- Green, T., Nash, J.A. and Mainwaring, G.(1986[b]). Methylene chloride (dichloromethane): In vitro metabolism in rat, mouse and hamster liver and lung fractions and in human liver fraction. Imperial Chemical Industries PLC. Central Toxicology Laboratory Report No: CTL/R/879.
- Green, T., Provan, W.M. and Gowans, N.(1987[a]). Methylene chloride (dichloromethane): In vivo inhalati pharmacokinetics in $B_6C_3F_1$ mice using stable isotopes. Imperial Chemical Industries PLC. Central Toxicology Report No: CTL/R/931.
- Green, T., Nash, J.A. and Hill, S.J.(1987[b]). Methylene chloride (dichloromethane): Glutathione-S-transferase metabolism in vitro in rat, mouse, hamster and human liver cytosol fractions. Imperial Chemical Industries PLC. Central Toxicology Laboratory Report No: CTL/R/934.
- Green, T., Nash, J.A., Hill, S.J. and Foster, J.R.(1987[c]). Methylene chloride (dichloromethane): The effects of exposure to 4000ppm on mouse lung enzymes. Imperial Chemical Industries PLC, Central Toxicology Laboratory Report No: CTL/R/935.
- Heppel, L.A., Neal, P.A., Perrin, T.L., Orr, N.L. and Porterfield, V.T.(1944). Toxicology of dichloromethane (methylene chloride): I Studies on effects of daily inhalation. J Ind Hyg Toxicol 26, 8-16.
- Hext, P.M., Foster, J. and Millward, S.W.(1986). Methylene chloride (dichloromethane): 10 day inhalation toxicity study to investigate the effects on rat and mouse liver and lungs. Imperial Chemic Industries PLC, Central Toxicology Laboratory Report No: CTL/P/1432.
- Howe, R.B.(1983). GLOBAL 83: An experimental program developed for the US Environmental Protection Agency as update to GLOBAL 82 : a computer program to extrapolate quantal animal toxicity data to low doses (May 1982). K S Crump and Co Inc, Rushton, LA. Unpublished.

- Kjellestrand, P., Holmquist, B., Jonsson, I., Romare, S. and Mansson, L.(1985). Effects of organic solvents on motor activity in mice. Toxicology 35, 35-46.
- Krijgsheld, K.R., Lowe, M.C., Minnaugh, E.G., Trush, M.A., Ginsburg, E. and Gram, T.E.(1983). Lung-selective impairment of cytochrome P-450 dependent monooxygenases and cellular injury by 1,1-dichloroethylene in mice. Biochem Biophys Res Comm 110, 675-681.
- Maltoni, C., Cotti, G. and Perino, G.(1986). Experimental research on methylene chloride carcinogenesis. Archives of research on industrial carcinogens Vol. IV. C Maltoni and M A Mehlman eds. Princeton Scientific Publishing Co. Princeton.
- NTP.(1986-Jan). NTP technical report on the toxicology and carcinogenesis studies of dichloromethane in F344/N rats and $B_6C_3F_1$ mice. NTP TR 306. Final Report.
- Ramsey, J.R. and Andersen, M.E.(1984). A physiological based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73, 159-175.
- Sato, A. and Nakajima, T.(1979). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. Brit J Ind Med 36, 231-234.
- Serota, D.G., Thakur, A.K., Ulland, B.M., Kirschman, J.C., Brown, N.M., Coots, R.H. and Morgareidge, K.(1986a). A two-year drinking-water study of dichloromethane in rodents. I. Rats. Fd Chem Toxicol 24, 951-958.
- Serota, D.G., Thakur, A.K., Ulland, B.M., Kirschman, J.C., Brown, N.M., Coots, R.H. and Morgareidge, K.(1986b). A two year drinking-water study of dichloromethane in rodents. II. Mice. Fd Chem Toxicol 24, 959-963.
- Smith, M.N., Greenberg, S.D. and Spjut, H.J.(1979). The Clara cell: A comparative ultrastructural study in mammals. Am J Anat 155, 15-30.
- Thomas, A.A. and Pinkerton, M.K.(1972). Effects of low level dichloromethane exposure on the spontaneous activity of mice. Armstrong Medical Research Laboratory, AMRL-TR-72-130. Paper No: 14, 223-238.
- Ward, R.C., Travis, C.C., Hetrick, D.M., Andersen, M.E. and Gargas, M.L.(1988). Pharmacokinetics of tetrachloroethylene. Toxicol Appl Pharamcol 93, 108-117.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 1

PHYSIOLOGICAL PARAMETERS

	Mouse	Rat	Hamster	Human
Weights (kg)				
Body	0.025	0.25	0.125	70
Lung ($x10^3$)	0.29	2.92	1.47	772.0
Tissue Compartments	Percentage of bodyweight			
Fat	4.0	7.0	7.0	21.1
Slowly perfused	78.0	75.0	75.0	49.9
Liver	4.0	4.0	4.0	2.3
Richly perfused	5.0	5.0	5.0	4.9
Partition Coefficients				
Blood/air	16.6	16.6	22.5	9.7
Fat/blood	5.5	5.5	4.1	8.8
Slowly perfused/blood	0.3	0.3	0.2	0.5
Liver/blood	0.6	0.5	0.4	0.7
Lung/blood	0.4	0.5	0.3	0.6
Richly perfused/blood	0.4	0.5	0.3	0.7

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 3

A COMPARISON OF EXPERIMENTAL DATA AND MODEL PREDICTIONS FOR THE MOUSE

Model predictions were obtained using the metabolic rate constants given in Tables 4 and 5.

Dose (ppm)	B.W. (kg)	Blood Levels of CH_2Cl_2 (mg/l)		Rate of Metabolism (mg/hr)			
				Cytochrome P-450		Glutathione-S-transferase	
		Predicted	Exptl	Predicted	Exptl	Predicted	Exptl
100	0.023	4.7	ND	0.42	0.37	0.12	0.13
500	0.023	15.2	5.6±3.3	0.44	0.44	0.31	0.30
4000	0.025	67.9	56.2±6.7	0.47	0.48	0.91	0.96

ND - Not determined.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 2

THE EFFECT OF EXERCISE ON HUMAN PHYSIOLOGICAL PARAMETERS[a]

A - Ventilation and cardiac output.

	Work Rate (Watts)			
	Rest	50	100	150
Alveolar ventilation l/min	4	17-22	31-36	36-46
Perfusion rate l/min	5	7-12	12-15	15-18
Perfusion-ventilation ratio	1.3	0.4-0.5	0.4-0.4	0.4-0.4

B - Distribution of blood flow l/min

	Rest	50	100	150
Lungs	5.0	10.0	14.0	17.0
Kidney	1.2	1.1	0.9	0.8
Liver (spleen, intestine)	1.6	1.6	1.3	0.9
Brain	0.7	1.1	1.15	1.2
Muscle, skin	1.1	5.0	9.0	12.0
Fat	0.15	0.45	0.7	0.5

a - data taken from Astrand (1983).

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 5

METABOLIC CONSTANTS FOR THE GLUTATHIONE-S-TRANSFERASE PATHWAY

	Vmax (mg/hr)	Bodyweight (kg)	Vmax (mg/hr/kg)	Km mg l^{-1}
In vitro hepatic constants				
Mouse	30.2	0.025	1208	7310
Rat	22.7	0.250	90.8	1785
Hamster	3.27	0.125	26.2	1785
Human	239	70.0	3.4	1785
In vivo scaled constants				
Mouse[a]	1.74	0.025	69.6	55
Rat[b]	1.31	0.250	5.22	13
Hamster[b]	0.19	0.125	1.51	13
Human[b]	13.74	70.0	0.20	13

a - measured experimentally in vivo.

b - scaled from the mouse in the ratio of the in vitro constants.

See also Appendix 2.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 4

METABOLIC CONSTANTS FOR THE CYTOCHROME P-450 PATHWAY

	Vmax (mg/hr)	Bodyweight (kg)	Vmax (mg/hr/kg)	Km mg l^{-1}
In vitro hepatic constants				
Mouse	0.31	0.025	12.4	67
Rat	0.68	0.250	2.7	73
Hamster	0.85	0.125	6.8	241
Human	98.0	70.0	1.4	70
In vivo scaled constants				
Mouse[a]	0.371	0.025	14.8	0.36
Rat[b]	0.81	0.250	3.25	0.39
Hamster[b]	1.02	0.125	8.13	1.29
Human[b]	117.4	70.0	1.68	0.38

a - measured experimentally in vivo.
b - scaled from the mouse in the ratio of the in vitro constants.
See also Appendix 2.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 7

A COMPARISON OF EXPERIMENTAL HUMAN DATA WITH MODEL PREDICTIONS

A - Studies by DiVincenzo and Kaplan (1981)

Volunteers exposed to 50-200ppm for 7½hr. [a]

Exposure (ppm)	Pulmonary Uptake (mmole)		Post Exposure Elimination of CH_2Cl_2 (mmole)		Cytochrome P-450 Metabolism			
					CO (mmole)		CO_2 (mmole)[b]	
	Exptl	Predicted	Exptl	Predicted	Exptl	Predicted	Exptl	Predicted
50	5.54	5.59	0.19	0.13	1.32	2.33	ND	3.09
100	10.70	10.49	0.56	0.35	2.66	4.31	ND	5.71
150	15.38	14.48	0.75	0.69	4.95	5.83	ND	7.73
200	21.07	17.70	1.02	1.17	6.12	6.95	ND	9.21

a - a bodyweight of 75kg was used.
b - calculated according to the ratio of CO:CO_2 obtained from the cytochrome P-450 pathway in mice exposed at 100ppm.
ND - not determined experimentally.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 6

THE USE OF IN VITRO SCALING TO PREDICT THE IN VIVO METABOLISM OF METHYLENE CHLORIDE IN THE RAT

In vivo metabolic rate constants obtained in mice were scaled according to in vitro rate constants measured in mice and rats. The rate constants obtained for the rat in this way were used to predict the metabolism of methylene chloride in the rat in vivo.

Dose (ppm)	Blood Levels of Methylene Chloride		Metabolism by			
			Cytochrome P-450		Glutathione-S-transferase	
	Exptl mg/l	Predicted mg/l	Exptl[b] (mg/hr)	Predicted (mg/hr)	Exptl[b] (mg/hr)	Predicted (mg/hr)
500[a]	6 (3-11)	17.8	1.27	1.06	0.36	0.75
4000	218	214	0.72	0.94	1.03	1.14

a - because of the wide experimental variation at this dose level the range of blood levels is also given.

b - calculated according to the ratio of CO:CO_2 obtained from the cytochrome P-450 pathway in mice exposed at 500 and 4000ppm.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 8

A COMPARISON OF EXPERIMENTAL HUMAN DATA WITH MODEL PREDICTIONS
STUDIES BY ASTRAND et al (1975)

The uptake of methylene chloride was measured in a volunteer exposed at rest to 250ppm for 30 min followed by a further 30 min at 500ppm. Following a break of 20 min the experiment was repeated during exercise at an intensity of 50W. The experimentally measured uptake is compared with that predicted by the model.

Exposure (ppm)	Time (min)	Exercise (W)	Dose Given (mg)	Uptake (mg)	Predicted (mg)
250	0-30	Rest	201	109	102
500	30-60	Rest	432	216	186
0	60-75	Rest	-	-	-
0	75-80	50	-	-	-
250	80-110	50	537	198	209
500	110-140	50	1203	376	359

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 7 - continued

B - Studies by Engstrom and Bjurstrom (1977)

Volunteers exposed to 750ppm for 1hr (50W work).

	Group I Subjects		Group II Subjects	
Bodyweight (kg)	71.9 ± 1.8		98.5 ± 10.8	
	Exptl	Predicted	Exptl	Predicted
Uptake (mg)	1116±34	1093	1446±110	1239
Venous Blood Concentration (mg/l)	15.6±0.8	19.5	14.6±1.0	19.3

METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 10

RISKS OF LIVER OR LUNG TUMOURS IN MAN FOLLOWING EXPOSURE TO METHYLENE CHLORIDE

TLV (ppm)	Liver (Quadratic Multistage)	Liver (Weibull)	Lung (Quadratic Multistage)	Lung (Weibull)	Lung + Liver (Quadratic Multistage)	Lung + Liver (Weibull)
10	4.02×10^{-10}	$<1 \times 10^{-17}$	6.12×10^{-9}	3.55×10^{-12}	6.52×10^{-9}	3.55×10^{-12}
20	2.30×10^{-9}	$<1 \times 10^{-17}$	2.88×10^{-8}	3.05×10^{-11}	3.11×10^{-8}	3.05×10^{-11}
30	7.20×10^{-9}	$<1 \times 10^{-17}$	7.27×10^{-8}	1.11×10^{-10}	7.99×10^{-8}	1.10×10^{-10}
40	1.74×10^{-8}	$<1 \times 10^{-17}$	1.41×10^{-7}	2.77×10^{-10}	1.58×10^{-7}	2.77×10^{-10}
50	3.63×10^{-8}	$<1 \times 10^{-17}$	2.36×10^{-7}	5.65×10^{-10}	2.72×10^{-7}	5.65×10^{-10}
60	6.76×10^{-8}	$<1 \times 10^{-17}$	3.59×10^{-7}	1.01×10^{-9}	4.26×10^{-7}	1.01×10^{-9}
70	1.15×10^{-7}	$<1 \times 10^{-17}$	5.10×10^{-7}	1.65×10^{-9}	6.25×10^{-7}	1.65×10^{-9}
80	1.82×10^{-7}	$<1 \times 10^{-17}$	6.89×10^{-7}	2.51×10^{-9}	8.71×10^{-7}	2.51×10^{-9}
90	2.69×10^{-7}	$<1 \times 10^{-17}$	8.96×10^{-7}	3.61×10^{-9}	1.16×10^{-6}	3.61×10^{-9}
100	3.77×10^{-7}	$<1 \times 10^{-17}$	1.13×10^{-6}	4.96×10^{-9}	1.50×10^{-6}	4.96×10^{-9}
150	1.20×10^{-6}	$<1 \times 10^{-17}$	2.59×10^{-6}	1.57×10^{-8}	3.78×10^{-6}	1.57×10^{-8}
200	2.35×10^{-6}	1.39×10^{-17}	4.40×10^{-6}	3.28×10^{-8}	6.75×10^{-6}	3.28×10^{-8}
250	3.70×10^{-6}	6.94×10^{-17}	6.41×10^{-6}	5.54×10^{-8}	1.01×10^{-5}	5.54×10^{-8}
300	5.15×10^{-6}	1.94×10^{-16}	8.55×10^{-6}	8.25×10^{-8}	1.37×10^{-5}	8.25×10^{-8}
350	6.66×10^{-6}	4.30×10^{-16}	1.08×10^{-5}	1.13×10^{-7}	1.74×10^{-5}	1.13×10^{-7}
400	8.19×10^{-6}	8.05×10^{-15}	1.30×10^{-5}	1.47×10^{-7}	2.12×10^{-5}	1.47×10^{-7}
450	9.73×10^{-6}	1.36×10^{-15}	1.52×10^{-5}	1.84×10^{-7}	2.50×10^{-5}	1.84×10^{-7}
500	1.13×10^{-5}	2.11×10^{-15}	1.75×10^{-5}	2.32×10^{-7}	2.88×10^{-5}	2.23×10^{-7}

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

TABLE 9

THE CORRELATION BETWEEN INTERNAL DOSE AND TUMOUR INCIDENCE

The internal dose calculated in the liver, lungs and Clara cells of each animal species is compared with the results of the appropriate 2 year cancer study. Internal dose is calculated for a 24hr period at the start of which the animal is exposed for 6hr at the dose levels shown.

Species	External Dose (ppm)	Internal Dose (mg/l) P-450	Internal Dose (mg/l) GST	Tumour Incidence %
Liver				
Mouse[a]	2000	2204	3755	33
	4000	2285	4923	83
Rat[a]	2000	745	837	0
	4000	833	1012	0
Hamster[b]	1500	1502	232	0
	3500	1612	265	0
Lung				
Mouse[a]	2000	2277	420	63
	4000	2405	542	85
Rat[a]	2000	796	86	0
	4000	884	104	0
Hamster[b]	1500	1527	23	0
	3500	1636	27	0
Clara cells[c]				
Mouse[a]	2000	0	4282	63
	4000	0	5501	85

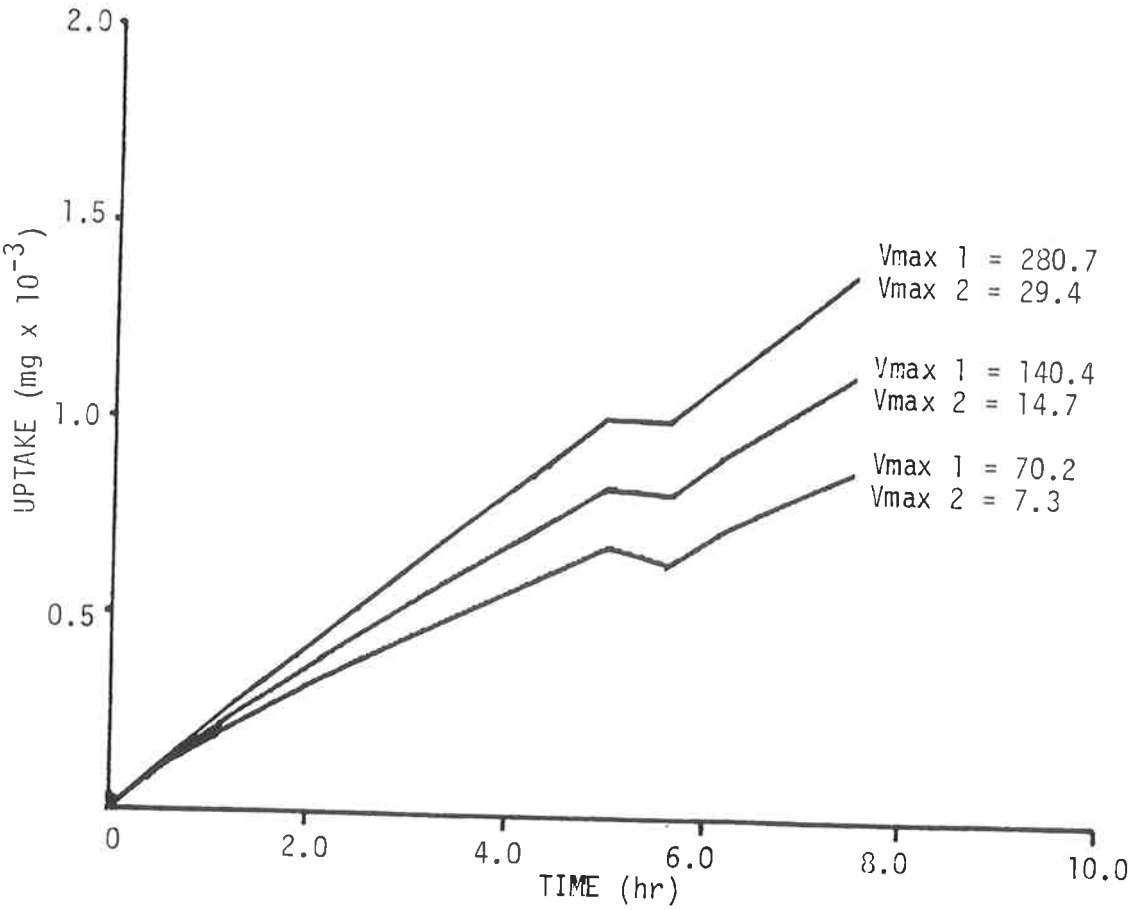
a - NTP 1986
b - Burek et al, 1984.
c - calculated using a model containing a separate Clara cell compartment.
P-450 - Cytochrome P-450 pathway.
GST - Glutathione-S-transferase pathway.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 1 - continued

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY DIVINCENZO AND KAPLAN (1981) AT 50PPM AND 200PPM

(b) The influence of metabolic rate on uptake in a volunteer exposed to 200ppm methylene chloride.



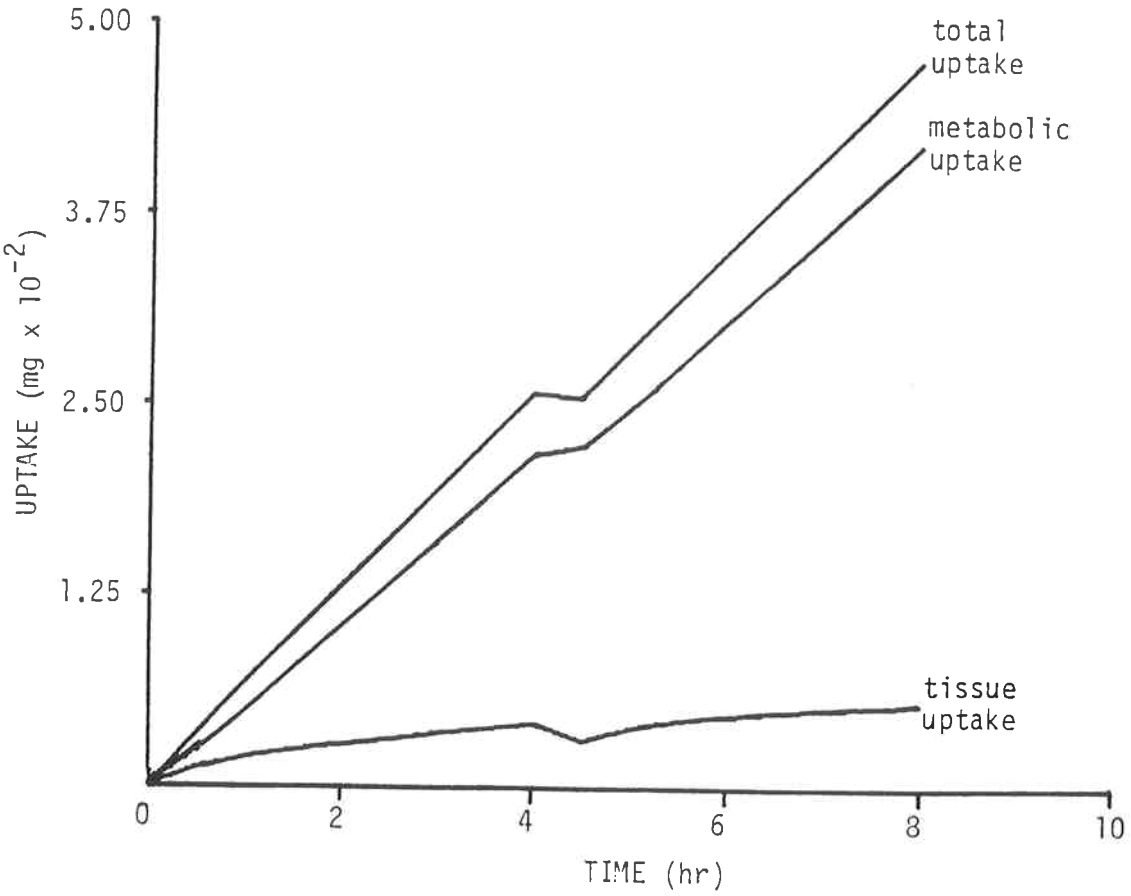
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 1

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY DIVINCENZO AND KAPLAN (1981) AT 50PPM AND 200PPM

A human volunteer was exposed to methylene chloride for 7½hr with a break of ½hr after 4hr exposure. The experimental and predicted values for uptake and metabolism are given in Table 7. The figures below show (a) the influence of metabolism and tissue uptake on the total uptake at 50ppm and (b) the influence of metabolic rate (Vmax) on uptake at 200ppm.

(a) The influence of metabolism and tissue uptake on total uptake.



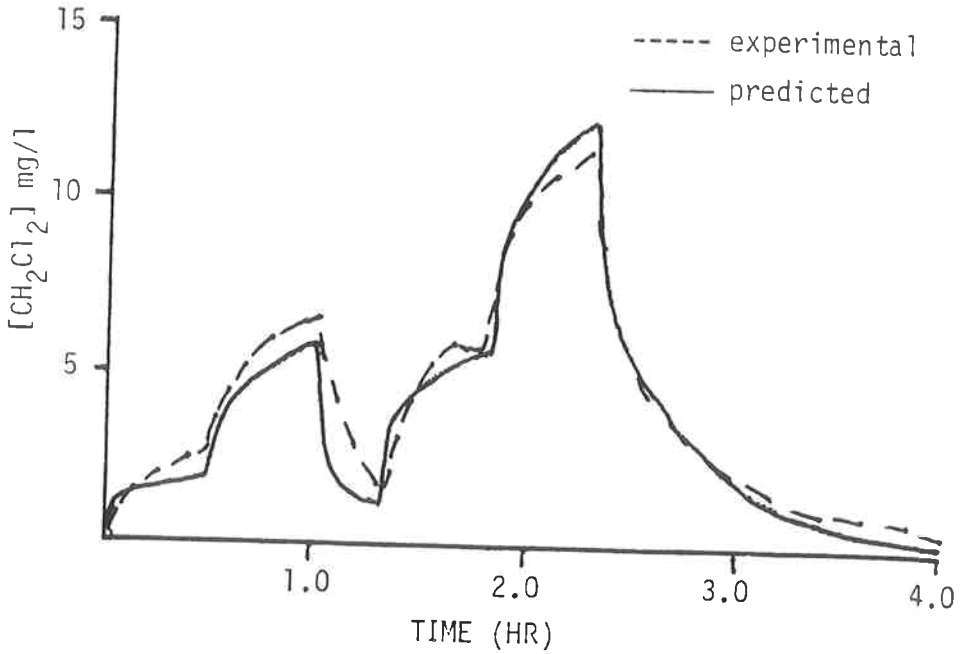
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 2 - continued

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY ASTRAND et al (1975) AT 250/500PPM

(b) Blood Levels

The details of the protocol are given on the previous page.



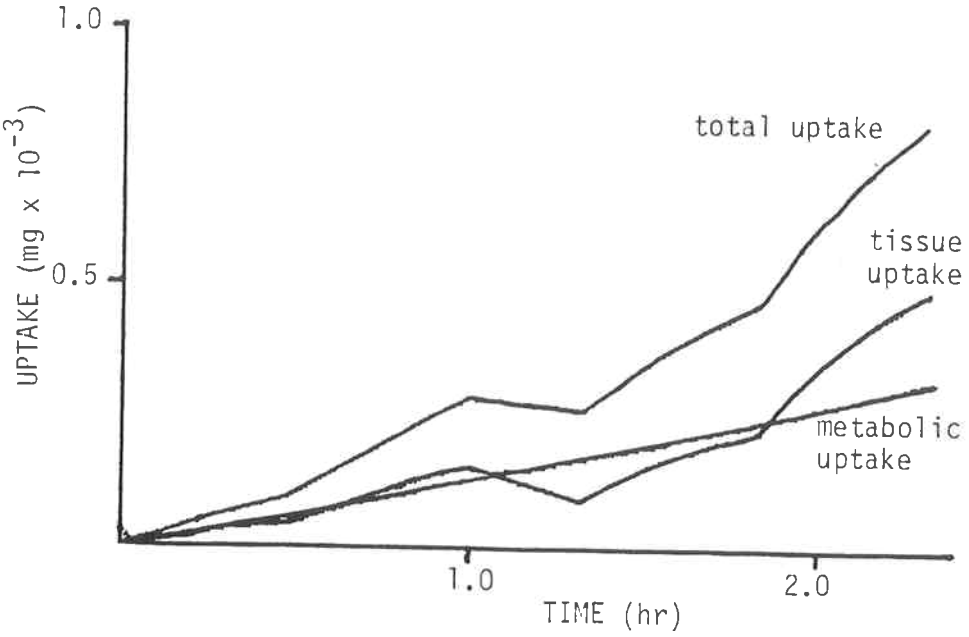
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 2

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY ASTRAND et al (1975) AT 250/500PPM

The uptake of methylene chloride was measured in a volunteer exposed at rest to 250ppm for 30min followed by a further 30mins at 500ppm. Following a break of 20min the experiment was repeated during exercise at an intensity of 50W. The total uptake in this experiment was 899mg. Details of the uptake for each 30min period are given in Table 8. The figures below shows (a) the influence of metabolism and tissue uptake on the total uptake and (b) the blood levels of methylene chloride during exposure.

(a) Tissue Uptake

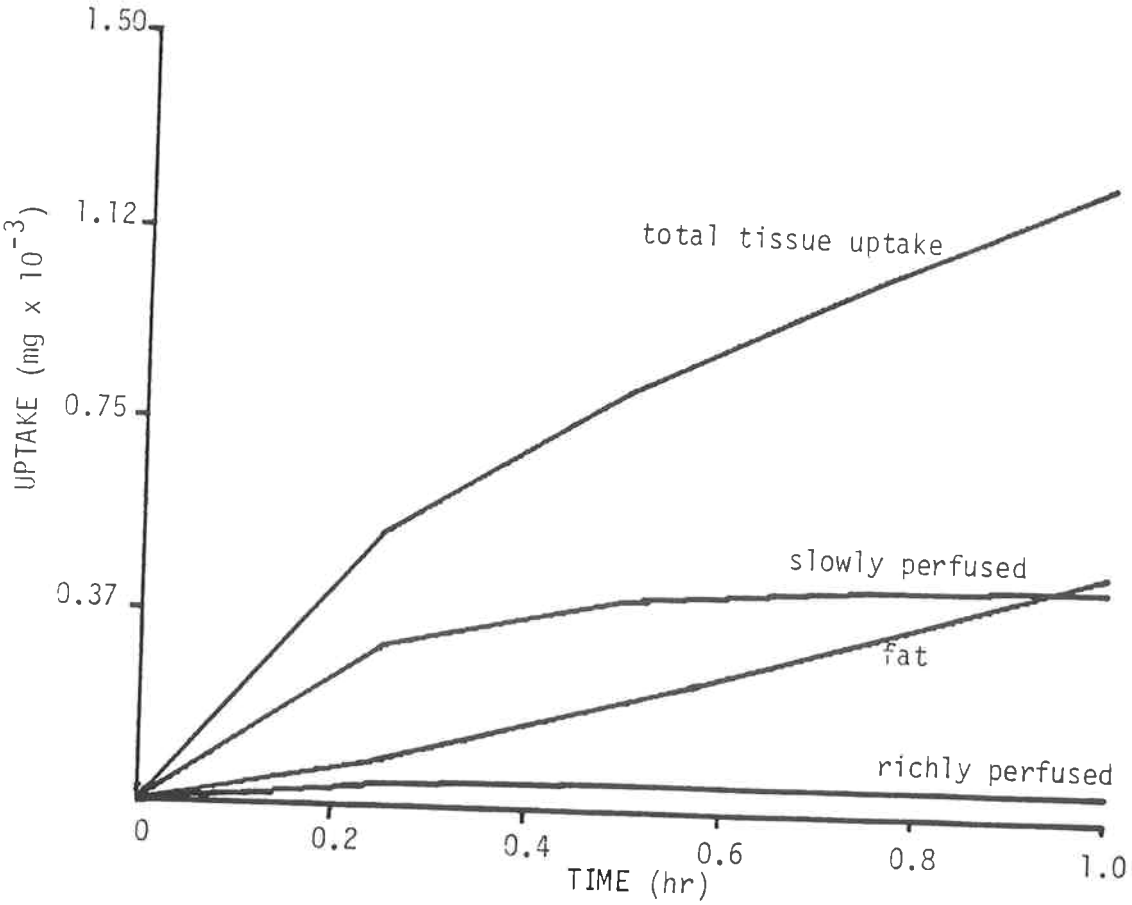


METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 3 - continued

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY ENGSTROM AND BJURSTROM (1977) AT 750PPM

(b) Tissue Distribution



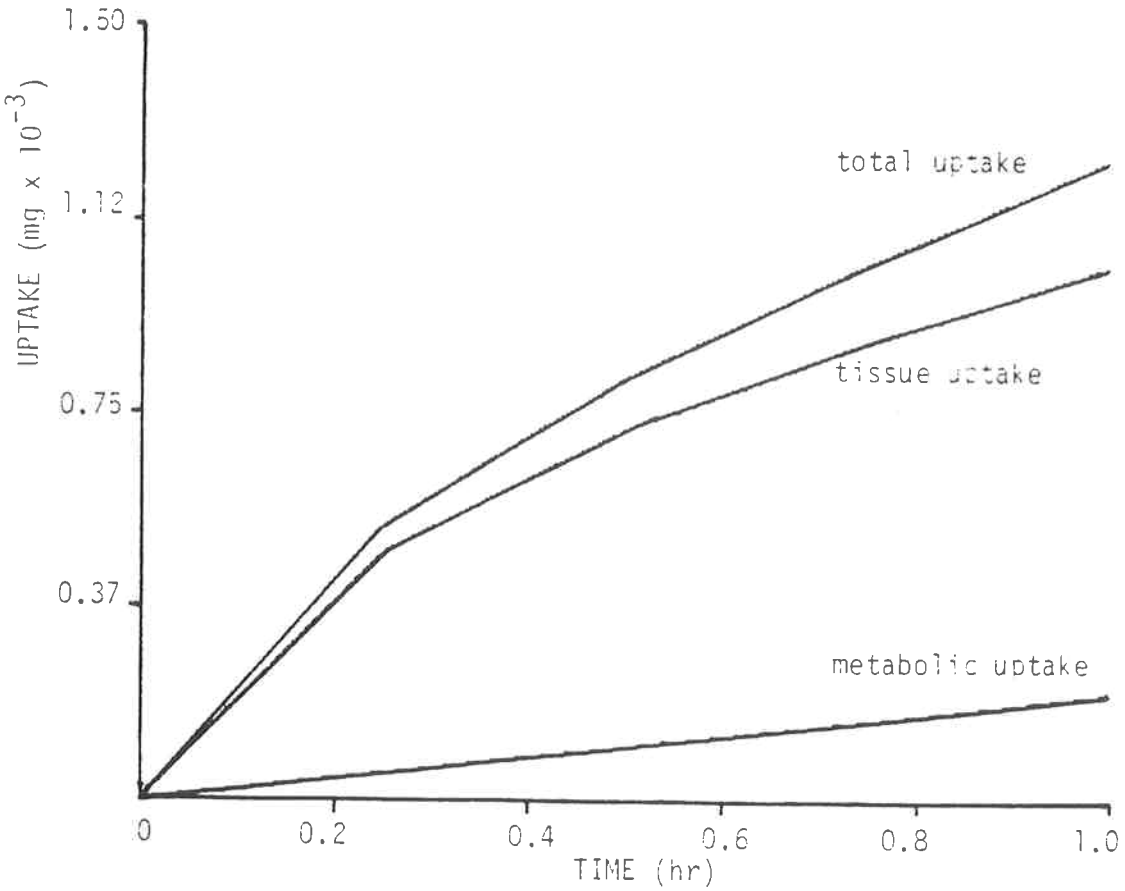
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 3

FACTORS INFLUENCING THE UPTAKE OF METHYLENE CHLORIDE IN HUMANS
STUDIES BY ENGSTROM AND BJURSTROM (1977) AT 750PPM

Volunteers were exposed for 1hr to 750ppm methylene chloride while performing light exercise (50W). Uptake and blood concentrations were measured (Table 7). The figures below shows (a) the influence of metabolism and tissue uptake on total uptake, and (b) the distribution of methylene chloride between tissue types.

(a) Tissue Uptake

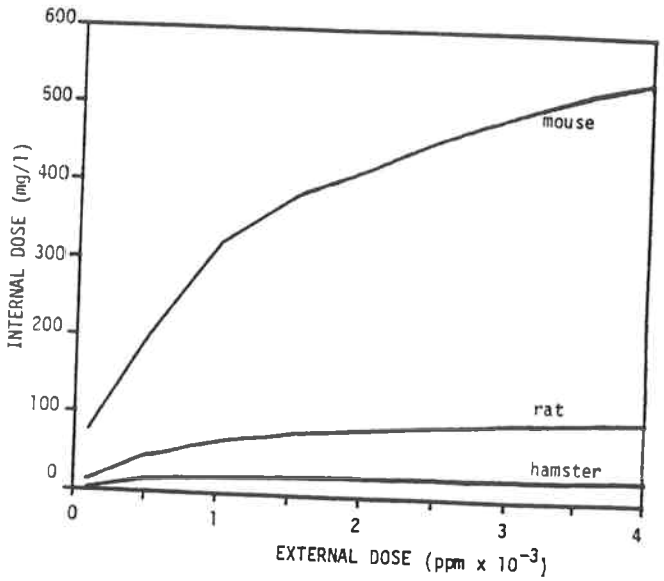


METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

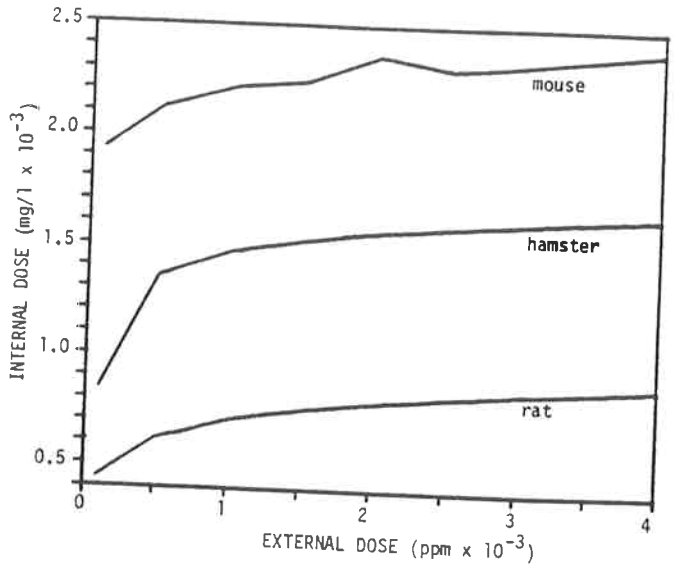
FIGURE 5

THE RELATIONSHIP BETWEEN INTERNAL DOSE AND EXTERNAL DOSE FOR THE LUNG IN ANIMALS (6HR EXPOSURES)

A - Glutathione-S-transferase metabolism



B - Cytochrome P-450 metabolism

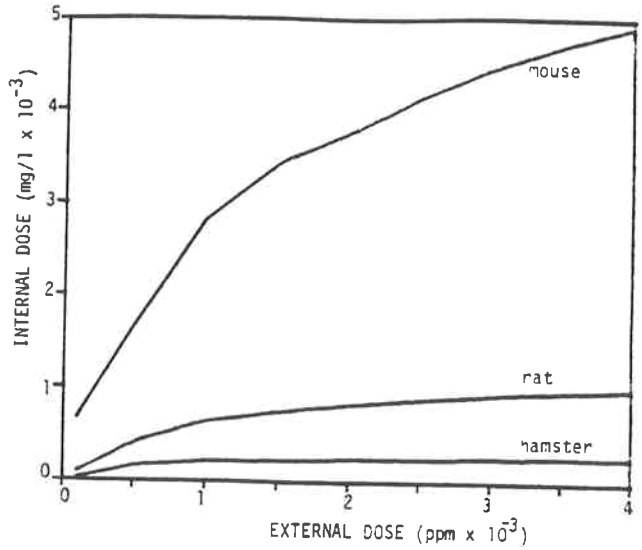


METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

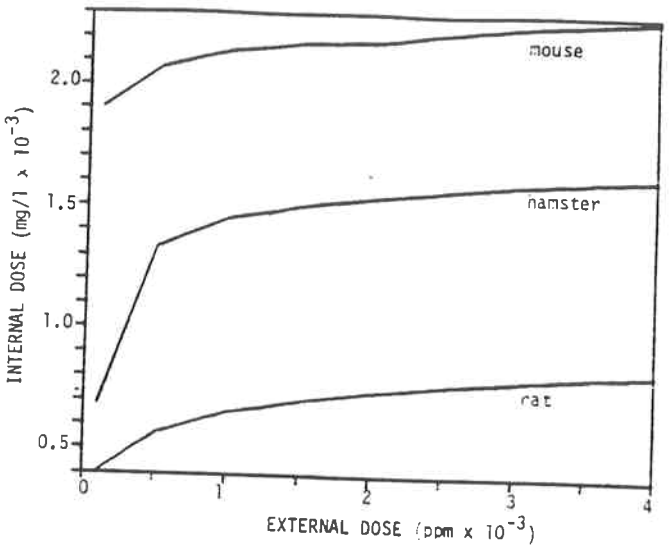
FIGURE 4

THE RELATIONSHIP BETWEEN INTERNAL DOSE AND EXTERNAL DOSE FOR THE LIVER IN ANIMALS (6HR EXPOSURES)

A - Glutathione-S-transferase metabolism



B - Cytochrome P-450 metabolism



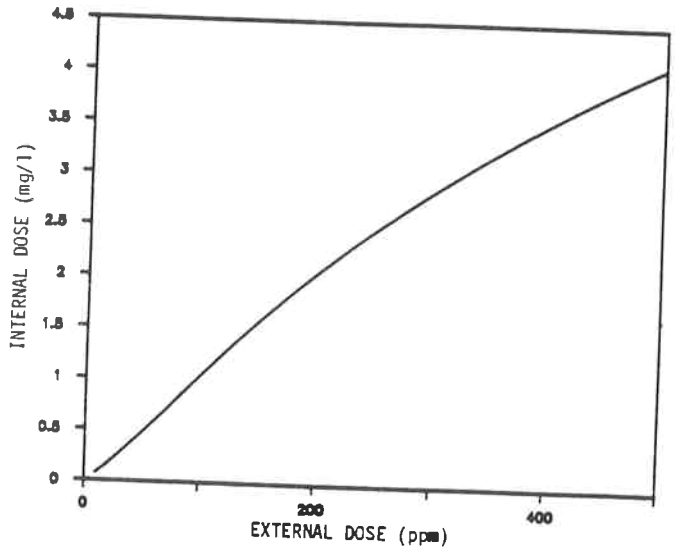
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 6 - continued

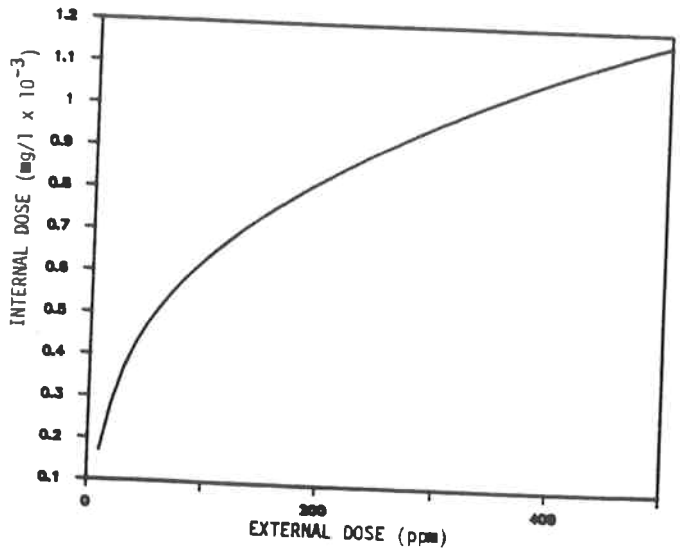
THE RELATIONSHIP BETWEEN INTERNAL DOSE AND EXTERNAL DOSE FOR HUMANS EXPOSED TO METHYLENE CHLORIDE

LUNG

A - Glutathione-S-transferase metabolism



B - Cytochrome P-450 metabolism



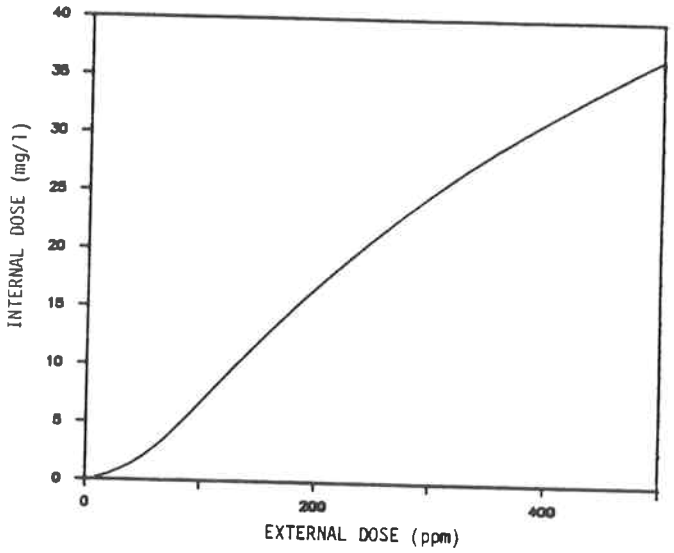
METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 6

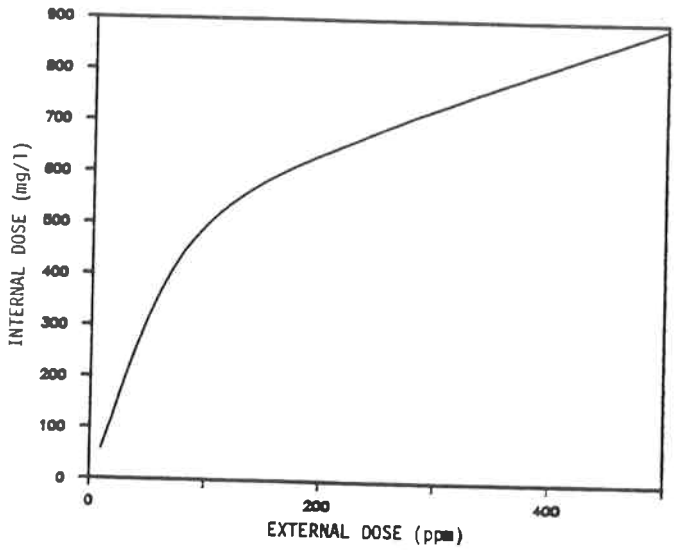
THE RELATIONSHIP BETWEEN INTERNAL DOSE AND EXTERNAL DOSE
FOR HUMANS EXPOSED TO METHYLENE CHLORIDE

LIVER

A - Glutathione-S-transferase metabolism



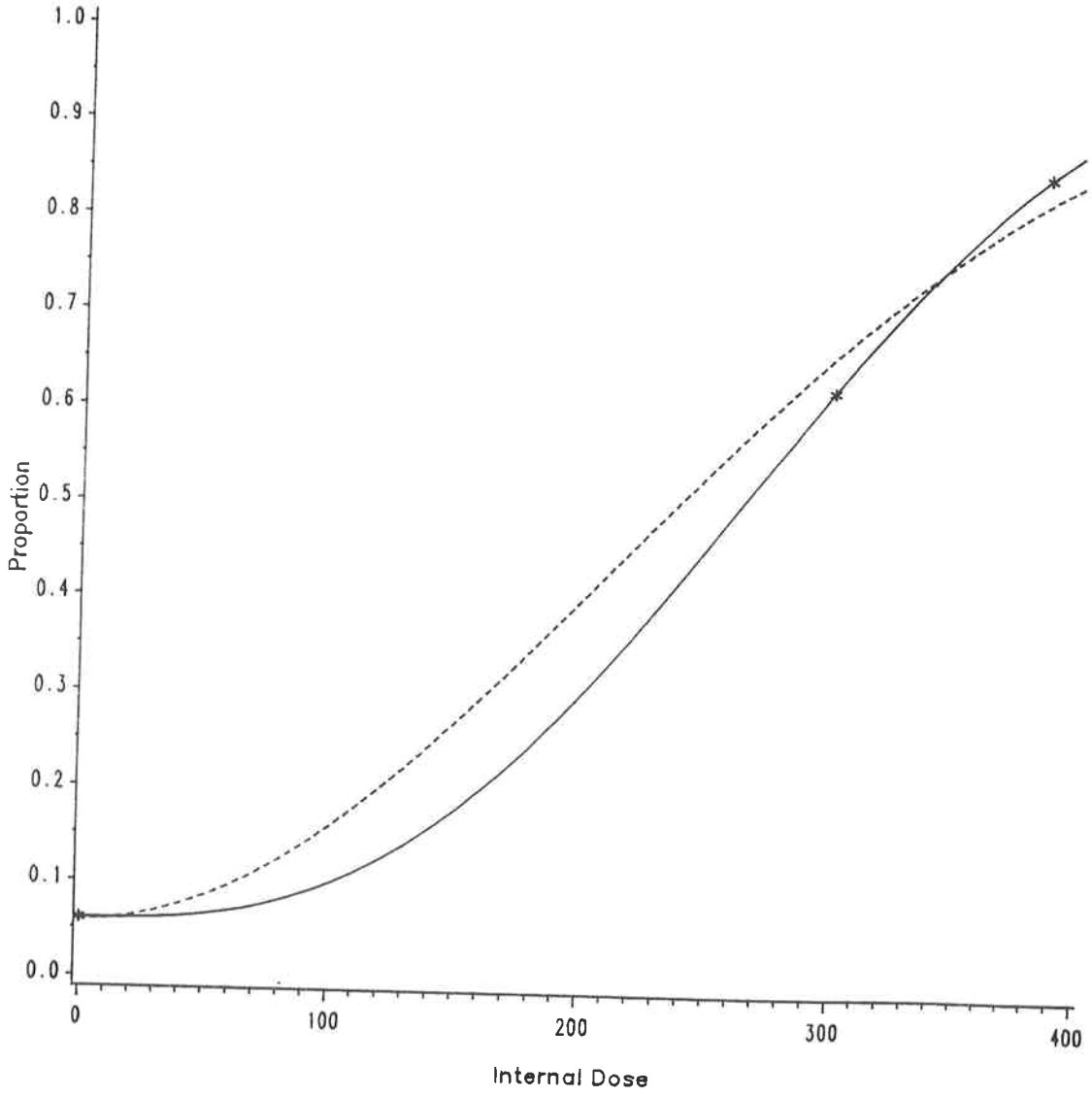
B - Cytochrome P-450 metabolism



METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 8

THE RELATIONSHIP BETWEEN INTERNAL DOSE AND THE TUMOUR INCIDENCES SEEN IN THE LUNGS OF FEMALE MICE IN THE NTP STUDY



* Observed proportion

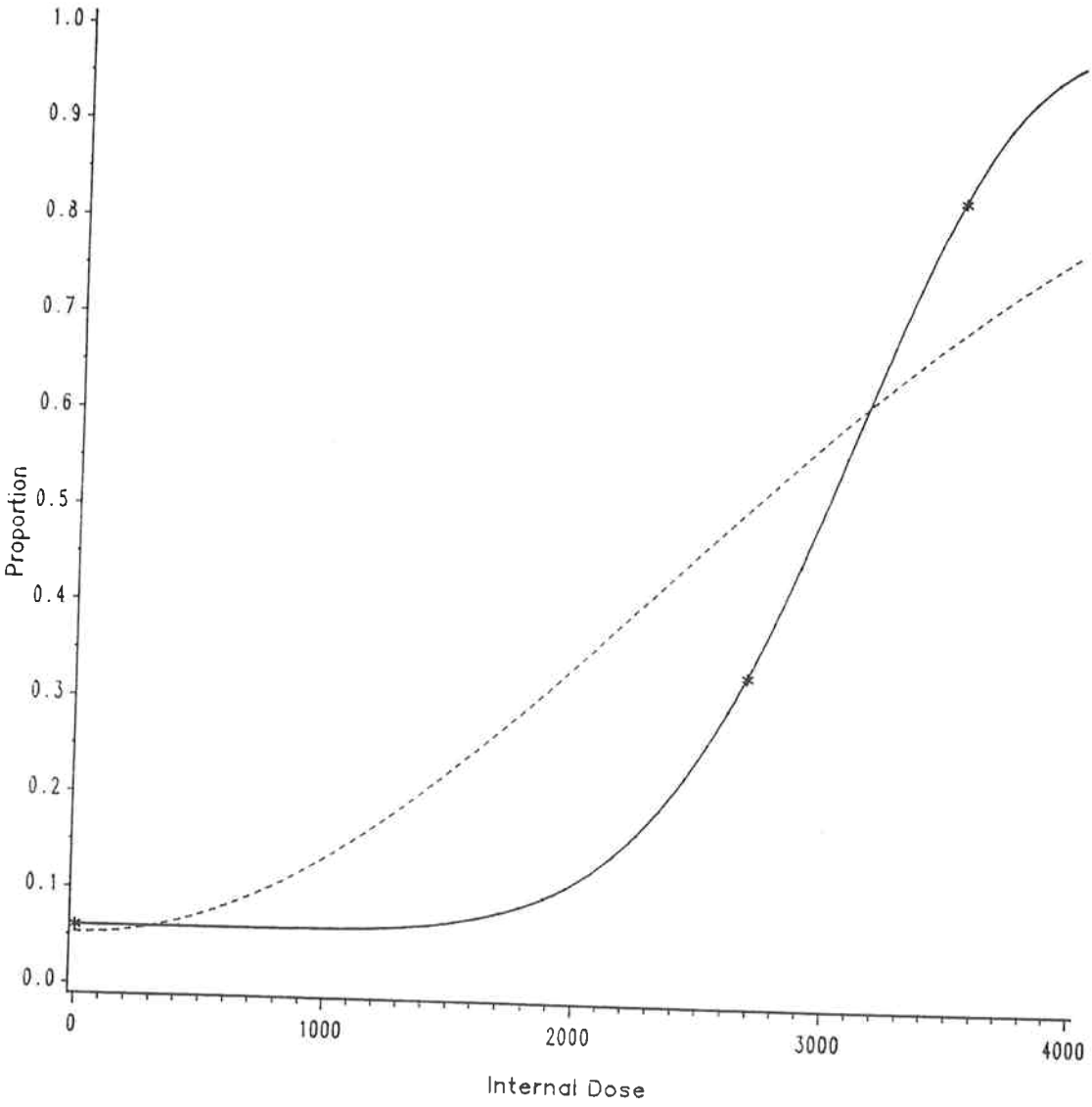
——— Expected proportion assuming a Weibull model

- - - - - - - - Expected proportion assuming a quadratic multistage model

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 7

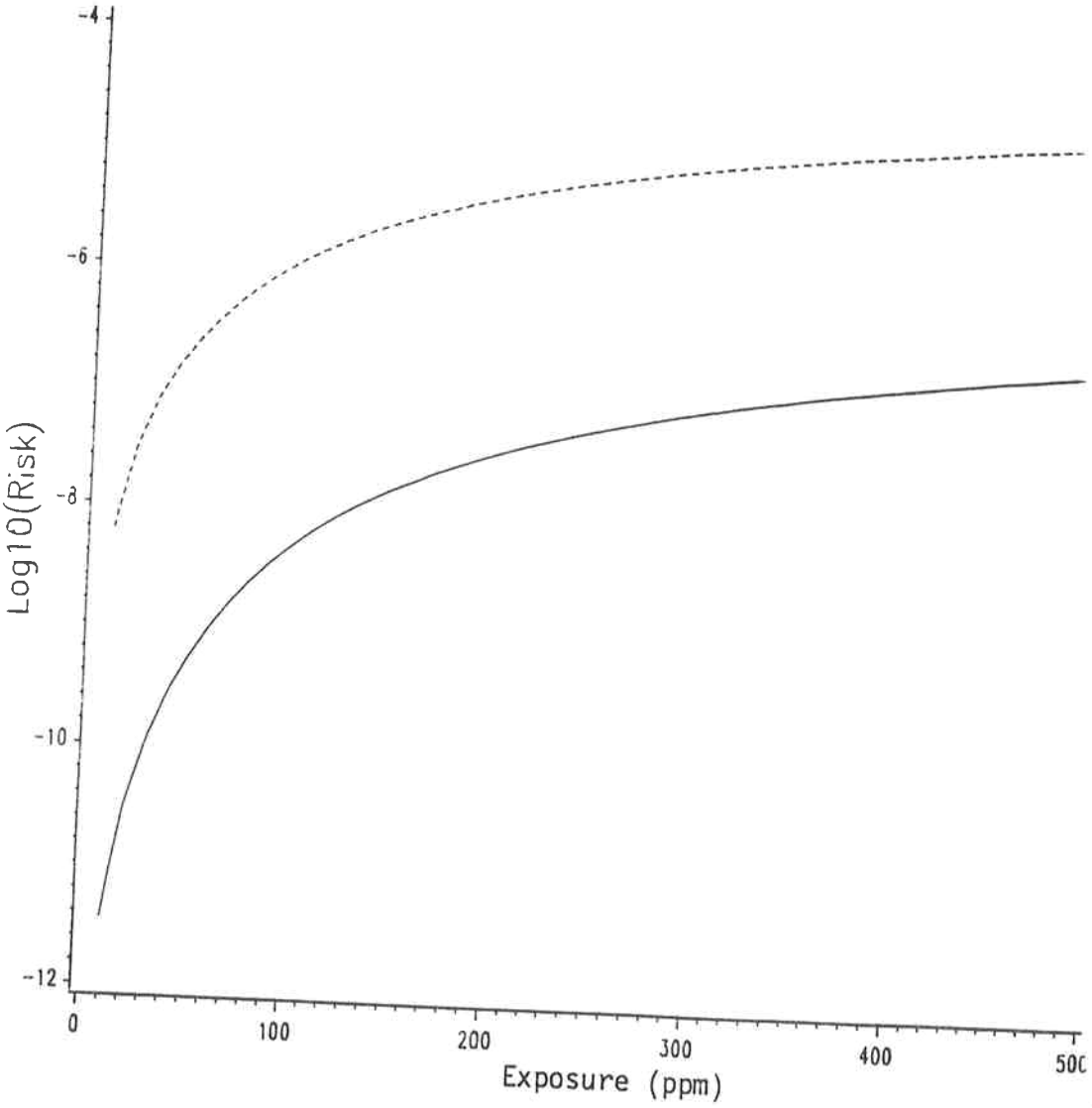
THE RELATIONSHIP BETWEEN INTERNAL DOSE AND THE TUMOUR INCIDENCES SEEN IN THE LIVERS OF FEMALE MICE IN THE NTP STUDY



METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 10

THE RELATIONSHIP BETWEEN RISK (MAXIMUM LIKELIHOOD ESTIMATE)
OF A LUNG TUMOUR AND EXPOSURE (ppm)



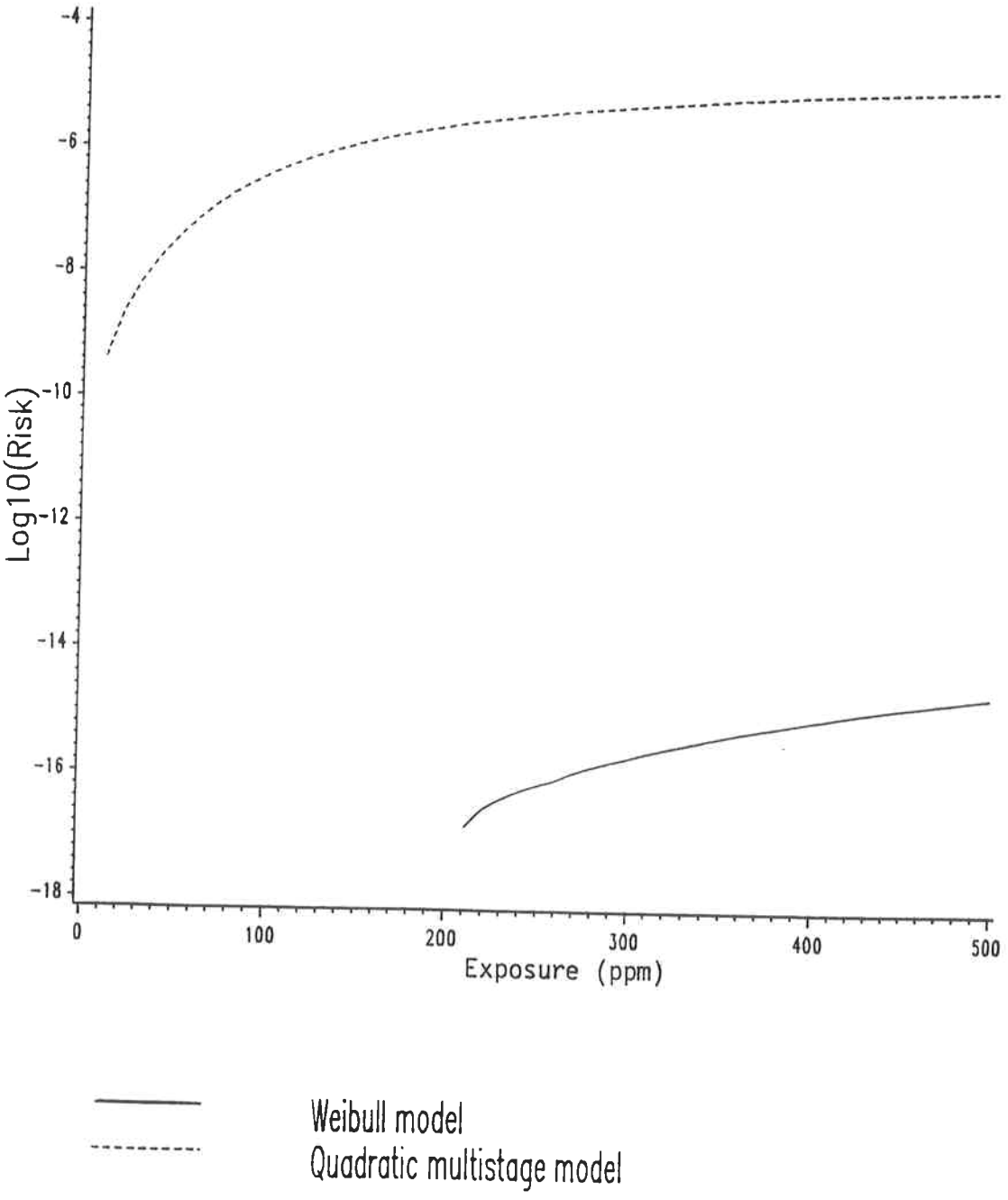
Weibull model

Quadratic multistage model

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

FIGURE 9

THE RELATIONSHIP BETWEEN RISK (MAXIMUM LIKELIHOOD ESTIMATE)
OF A LIVER TUMOUR AND EXPOSURE (ppm)



METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 2

THE METABOLIC RATE CONSTANTS USED IN THE MODEL

1. Cytochrome P-450 pathway

Km and Vmax have been measured for mouse, hamster and the rat (Green et al, 1986b). However the rate of metabolism by this pathway in human liver has only been measured at one concentration (15mM). At this single concentration the human metabolic rate is very similar to that of the rat which is in fact very close to the maximal rate. It has been assumed that this rate at 15mM is a maximal rate and hence that the Km for human liver is approximately equal to that in the rat which in turn is very similar to that in the mouse.

Km.rat = 73mg/l
Km.mouse = 67mg/l

Human Km assumed to be 70mg/l

2. The glutathione-S-transferase pathway

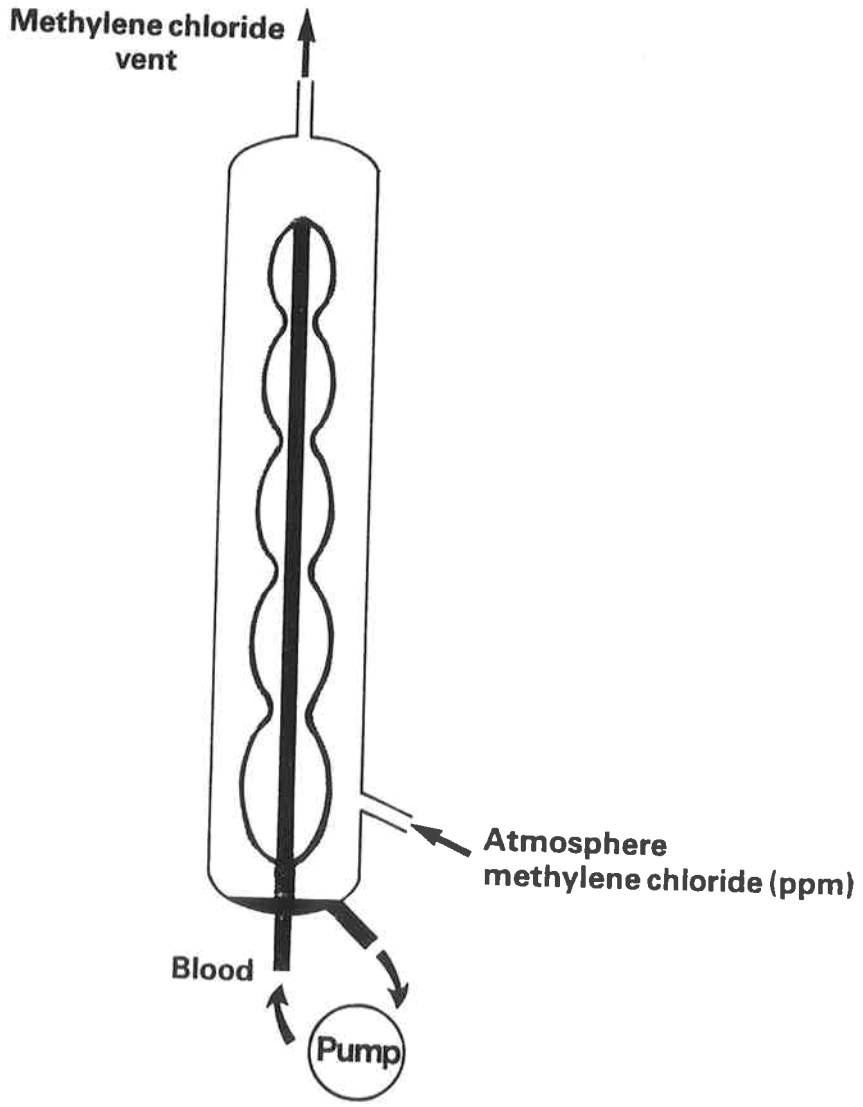
Km and Vmax values are available from one study assaying the rate of formation of formaldehyde from methylene chloride (Green et al, 1986b).

Species	Km (mM)	Vmax (n.moles/min/mg)
Mouse	86	36.4
Rat	21	2.9

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 1

APPARATUS FOR THE DETERMINATION OF BLOOD/AIR PARTITION COEFFICIENTS



The apparatus is maintained at 37°C. Equilibrium is established between the atmosphere of methylene chloride and the blood before blood samples are taken for analysis. The concentrations of methylene chloride in blood and in the atmosphere are used to calculate the partition coefficient.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 2 - continued

THE METABOLIC RATE CONSTANTS USED IN THE MODEL

The rates for either radioisotope assay have been used and where both are available the highest rates have been used.

Species	35mM Rate	Estimated Vmax (n.mol/min/mg)
Mouse	26.25	92.1
Rat	5.35	7.46
Hamster	1.65	-
Human	0.42	-

From the ratio of the rates at 35mM, to the maximal rate for the formaldehyde assay in rats and mice, the maximal rates have been estimated for the radiolabelled assay.

Vmax values have not been measured by any of the assays for either hamster or human liver. At the 35mM substrate concentration the hamster and human rates more closely resemble the rat than the mouse. Consequently the Km value for these two species has been assumed to be same as that in the rat (21mM). If this assumption is made it follows that the rate measurements at 35mM are approaching Vmax. The following metabolic constants have been used in the model.

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 2 - continued

THE METABOLIC RATE CONSTANTS USED IN THE MODEL

Human and hamster rates were lower and below the limit of detection of this assay. Rates were measured using radioisotopes (C-14, Cl-36) at a single substrate concentration of 35mM (Green et al, 1987b). The rates measured in rats and mice with this technique were higher than those measured with the formaldehyde assay due to losses of formaldehyde by further metabolism.

Species	35mM Rates (nmol/min/mg)		
	HCHO Assay	C-14 Assay	Cl-36 Assay
Mouse	10.37	26.25	17.20
Rat	2.08	5.35	N.A.
Hamster	N.D.	1.65	1.01
Human	N.D.	N.A.	0.42 ± 0.32

N.D. - Not detected
N.A. - Not available

METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 3

MAXIMUM LIKELIHOOD ESTIMATES AND CONFIDENCE LIMITS OF RISKS DERIVED FROM THE WEIBULL AND QUADRATIC MULTISTAGE MODELS SHOWN IN FIGURES 7 AND 8

The equation for the Weibull model is:-

$$P = 1 - \exp(-\gamma - \beta d^{\gamma})$$

which leads to

$$R = 1 - \exp(-\beta d^{\gamma})$$

The maximum likelihood estimates and upper confidence limits of β and γ are:-

Liver	$\beta = 5.3525 \times 10^{-22}$	$\beta^{*} = 1.0743 \times 10^{-14}$
	$\gamma = 5.838$	$\gamma^{*} = 3.835$
Lung	$\beta = 6.5444 \times 10^{-8}$	$\beta^{*} = 2.8988 \times 10^{-3}$
	$\gamma = 2.732$	$\gamma^{*} = 0.9935$
	$(\gamma = 6.188 \times 10^{-2})$	

The equation for the Quadratic Multistage Model is:-

$$P = 1 - \exp(-q0 - q_1 d - q_2 d^2)$$

The maximum likelihood estimates and upper confidence limits of q_1 and q_2 are:-

Liver	$q_1 = 0$	$q_1^{*} = 8.8289 \times 10^{-5}$
	$q_2 = 4.8623 \times 10^{-8}$	$q_2^{*} = 2.8860 \times 10^{-8}$
	$(q_0 = 5.407 \times 10^{-2})$	
Lung	$q_1 = 0$	$q_1^{*} = 3.0592 \times 10^{-3}$
	$q_2 = 5.9474 \times 10^{-6}$	$q_2^{*} = 0$
	$(q_0 = 6.062 \times 10^{-2})$	

METHYLENE CHLORIDE (DICHLOROMETHANE):
HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 2 - continued

THE METABOLIC RATE CONSTANTS USED IN THE MODEL

Species	Enzyme	Km (mM)	Vmax (nmol/min/mg)
Mouse	P-450	0.79	1.94
Rat	P-450	0.86	0.58
Hamster	P-450	2.83	1.85
Human	P-450	0.83	0.87
Mouse	GST	86	92.10
Rat	GST	21	7.46
Hamster	GST	21	1.65
Human	GST	21	0.42

METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 5

MEMBERS OF ECETOC SCIENTIFIC COMMITTEE

I F H PURCHASE (Chairman), Director, Central Toxicology Laboratory	ICI (Alderley Park)
M SHARRATT, (Vice Chairman), Senior Toxicologist	BP (Sunbury)
B BROECKER, Coordinator, Product-related Environmental Problems	HOECHST (Frankfurt)
H O ESSER, Head of Biochemistry, Agricultural Division	CIBA GEIGY (Basel)
P GELBKE, Head, Department of Toxicology	BASF (Ludwigshafen)
P A GILBERT, Head of Environmental Toxicology	Unilever (Port Sunlight)
U KORALLUS, Medical Director	BAYER (Leverkusen)
C LUNDBERG, Chief Toxicologist	NOBEL INDUSTRIES SWEDEN AB (Karlskoga)
R MILLISCHER, Chief Toxicologist	ATOCHEM (Paris La Defense)
H G NOSLER, Head, Coord. Centre for Enviromental Protection and Consumer Safety	HENKEL (Dusseldorf)
S PAGLIALUNGA, Head of Industrial Toxicology	MONTEDISON (Novara)
C L M POELS, Group Toxicology Division	SHELL (den Haag)
W F TORDOIR, Head of Occupational Health and Toxicology Division	SHELL (den Haag)
H VERSCHUUREN, Head, Departmen of Toxicology	DOW CHEMICAL (Horgen)

Responsible editor : D A Stringer, ECETOC
Avenue Louise 250, Bte 63 D-1988-3001/53
B-1050 Brussels, Belgium

METHYLENE CHLORIDE (DICHLOROMETHANE): HUMAN RISK ASSESSMENT USING EXPERIMENTAL ANIMAL DATA

APPENDIX 4

MEMBERSHIP OF ECETOC METHYLENE CHLORIDE STUDY GROUP

1. Dr M J L Clapp (Chairman), Imperial Chemical Industries PLC, Central Toxicology Laboratory
2. Professor H M Bolt, Dortmund University
3. Mr D G Farrar, Imperial Chemical Industries, Chemicals and Polymers Ltd
4. Dr T Hofmann, Hoechst
5. Professor J J Kolk, AKZO/Nijmegen University
6. Dr R Kuehnert, C W Huls
7. Dr R J Millischer, ATOCHEM
8. Drs C de Rooij, Solvay
9. Dr H G Verschuuren, Dow Chemical - Europe