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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.

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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

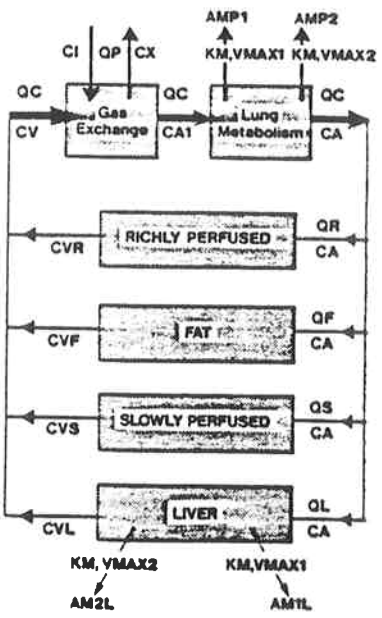
relationship to predict risk or doses associated with particular levels of risk. Standard assumptions are used by EPA to estimate the dose of methylene chloride during inhalational exposure of animals and the dose conversion to man is made on the basis of "equal response at equal doses per unit body 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