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ESTIMATING THE RISK OF HUMAN CANCER ASSOCIATED WITH EXPOSURE TO METHYLENE CHLORIDE

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Summary. - *Dichloromethane (methylene chloride, CH_2Cl_2) has been shown to significantly increase the incidence of malignant lung and liver tumors in $B_6C_3F_1$ mice inhaling high concentrations of CH_2Cl_2 vapor for the majority of their natural lifetime. CH_2Cl_2 is extensively metabolized in mammalian species through two competing pathways: (1) oxidation by the mixed function oxidase enzymes, and (2) conjugation with glutathione catalyzed by glutathione-S-transferase(s)(GST). Since elevated tumor incidences have not been observed in $B_6C_3F_1$ mice exposed to 1,1,1-trichloroethane, a halogenated solvent with similar physical-chemical properties (but only minor amounts of mammalian metabolism), it appeared that biologically reactive intermediates (BRIs) from one or both of the pathways of CH_2Cl_2 metabolism were involved in the tumorigenic process. Development of an integrated pharmacokinetic model incorporating quantitative measures of mammalian physiology, chemical solubility, and metabolic rate constants permitted formulation of a plausible hypothesis for the tumorigenic effects of CH_2Cl_2: namely that BRIs formed by the CH_2Cl_2/GST(s) may react with critical molecules in the target organs. This hypothesis is consistent with the dose-dependency, route-dependency, and species-specificity of CH_2Cl_2 for the induction of lung and liver tumors. Based on this hypothesis as well as* in vivo *and* in vitro *measurements of CH_2Cl_2 metabolism in humans, it was possible to prepare quantitative estimates of the cancer risk in human populations. Examination of these risk estimates indicates that development of quantitative procedures for describing the production of BRI in target tissues may cause significant changes in the levels of estimated risk.*

KEY WORDS: methylene chloride, cancer risk estimation, pharmacokinetic models, liver cancer, lung cancer, biologically reactive intermediates, cancer mechanisms.

Riassunto (La valutazione del rischio cancerogeno per l'uomo associato con l'esposizione al cloruro di metilene). - *E' stato dimostrato che il diclorometano (cloruro di metilene CH_2Cl_2) inalato ad alte dosi per la maggior parte del tempo vita, induce un significativo aumento dell'incidenza di tumori maligni del polmone in topi $B_6C_3F_1$. Il CH_2Cl_2 è efficacemente metabolizzato nei mammiferi attraverso due vie competitive: 1) ossidazione di ossidasi a funzione mista e 2) coniugazione con glutazione catalizzata da glutazione-S-transferasi (GST). Poiché nei topi $B_6C_3F_1$ esposti a 1,1,1-tricloroetilene, un solvente alogenato con simili proprietà fisico-chimiche (ma con un livello minimo di metabolizzazione nei mammiferi), non sono state osservate incidenze di tumore elevate, è apparso che nel processo di cancerogenesi sono coinvolti intermedi biologicamente reattivi (BRI) provenienti da una o entrambe le vie metaboliche del CH_2Cl_2. Lo sviluppo di un modello farmacocinetico integrato che incorpora dati di misura della fisiologia animale, della sulubilità chimica, e le costanti dei tassi metabolici, ha consentito la formulazione di un'ipotesi plausibile a riguardo degli effetti del CH_2Cl_2 : in sostanza, i BRI formati dal CH_2Cl_2/GST(s) possono reagire con molecole critiche nell'organo bersaglio. Questa ipotesi è consistente con la dose-dipendenza, la dipendenza dalla via metabolica, e la specie-specificità del CH_2Cl_2 rischio indica che lo sviluppo di procedure quantitative per la descrizione della produzione di BRI nei tessuti bersaglio può portare a cambiamenti significativi delle stime dei livelli di rischio.*

PAROLE CHIAVE: cloruro di metilene, stima del rischio cancerogeno, modelli farmacocinetici, intermediari biologicamente reattivi nel cancro del fegato e nel cancro del polmone, meccanismi del cancro.

Introduction

Dichloromethane (CH_2Cl_2, DCM) is a widely used industrial solvent. In addition to industrial applications such as film processing, it has been used for the preparation of consumer products such as cosmetics, decaffeinated coffee, and paint strippers. With such a wide potential for human exposure to DCM, the need for a good toxico-

logy database is obvious, and a number of acute, subchronic, and chronic studies have been conducted with this material. In general the results from these studies have been reassuring until the National Toxicology Program completed a long term inhalation bioassay of methylene chloride in rats and mice [1].

The results observed in the NTP inhalation study are inconsistent with those observed in another bioassay of DCM in $B_6C_3F_1$ mice reported by Serota *et al.* [2]. In the Serota study, DCM was administered in the drinking water and the incidences of lung and liver tumors in treated animals were not significantly different than control groups (Table 1, lung tumors not shown).

The obvious question raised by the NTP studies is whether human populations exposed to much lower concentrations of DCM vapor (or to low doses of DCM through other routes, such as drinking water) are likely to develop tumors similar to those seen in $B_6C_3F_1$ mice. While there are many biological factors which must be considered in answering this question, one of the primary considerations involves the delivery of toxic species to the target tissues in the animals. We have recently developed a physiologically-based pharmacokinetic (PB–PK) model capable of quantitatively describing the delivery of DCM and its metabolites to target organs [3], and have suggested that this model would be useful for the preparation of quantitative risk estimations with DCM.

Results and discussion

Before a realistic quantitative risk estimation can be performed, it is necessary to consider information on the mechanism(s) by which DCM influences the tumorigenic process in the $B_6C_3F_1$ mouse.

Dihalomethanes, including DCM, are metabolized by two major pathways: (1) an oxidative pathway [4] that appears to yield CO as well as considerable amounts of CO_2, [5] and (2) a glutathione-dependent pathway [6] which produces formaldehyde and CO_2 but no CO (Fig. 1). Potentially reactive intermediates are formed in each of the metabolic pathways for DCM: formyl chloride in the oxidative pathway, and formaldehyde and chloromethyl glutathione in the conjugative pathway.

Distribution of DCM metabolism between these two pathways is dose-dependent. The oxidative (MFO) pathway is a high-affinity limited-capacity pathway which saturates at relatively low atmospheric concentrations (200-500 ppm). In contrast, the conjugative (GSH) pathway has lower affinity for DCM, but does not appear to saturate at experimentally accessible concentrations (< 5000 ppm). Thus at low concentrations, the MFO pathway accounts for most of the DCM metabolized, but as exposure concentrations are increased above the MFO saturation level, disproportionate increases in the amount of DCM metabolized by the secondary GSH pathway are seen [3].

With this knowledge of DCM metabolism, three different hypotheses can be formulated to account for the tumorigenicity of DCM in the $B_6C_3F_1$ mouse:

1. - Tumorigenicity results from production of biologically reactive intermediates (BRIs) by the conjugative (GSH) pathway in target tissues.

2. - Tumorigenicity results from production of BRIs by the oxidative (MFO) pathway in target tissues.

3. - Tumorigenicity results from the presence of parent chemical in the target tissues.

Since the chemical reactivity of DCM is very low, it is unlikely that parent material would bond directly to biological macromolecules. Thus it is unlikely that the parent chemical could cause genetic alterations (through covalent binding to DNA) similar to those produced by many other chemical carcinogens [7].

This raises the question as to whether some physical property of the parent material (e.g. alteration of membrane permeability) might be responsible for the tumorigenicity of DCM. We consider this to be unlikely, because another solvent with physical properties similar to DCM (1,1,1-trichloroethane) has recently been tested in a long term inhalation bioassay in $B_6C_3F_1$ mice and it failed to induce either lung or liver tumors. The primary difference between DCM and 1,1,1-trichloroethane is that 1,1,1 trichloroethane is very poorly metabolized by mammalian species.

Collectively these observations suggest that the tumorigenicity of DCM probably resulted from the production of reactive metabolites of DCM rather than from a physical effect of the solvent itself. Consequently, we have

Table 1. - *Liver tumor incidences (combined adenomas and carcinomas) in $B_6C_3F_1$ mice as reported by Serota* et al. *[2] in a chronic drinking water study of DCM for chronic toxicity and/or carcinogenicity (lung tumor incidences in treated groups were not significantly different from controls; data not shown)*

	mg/kg/day from water					
	0	0	60	125	185	250
MALE MICE Liver tumors	18%	20%	28%	30%	31%	28%
FEMALE MICE Liver tumors	6%	6%	4%	4%	10%	6%

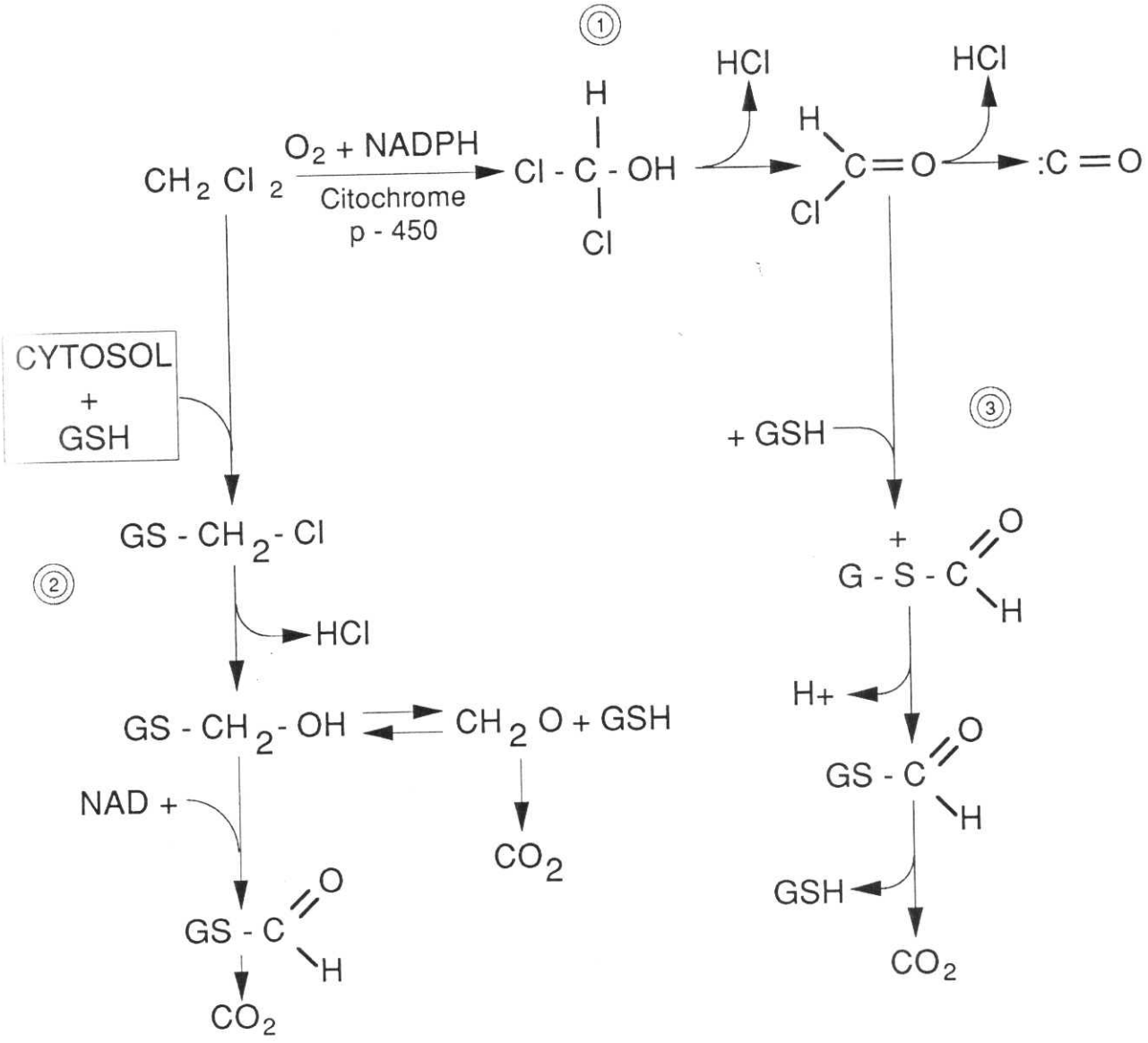

Fig. 1. - Major metabolic pathways of dialomethanes.

concluded that DCM is unlikely to directly influence tumor frequencies in the lung and liver of $B_6C_3F_1$ mice and have rejected hypothesis (3). This leaves the task of determining whether BRI's produced by MFO (hypothesis 2) or GSH transferase (hypothesis 1) are involved in the tumorigenic action of DCM.

The availability of a mathematical model capable of quantitatively describing the production of metabolites in target tissues by either the MFO or GSH pathway [3] provides an opportunity to test these hypotheses against the results obtained in the two long term bioassays of DCM. For this purpose, predicted amounts of DCM metabolism produced by each pathway (mg equivalents of DCM metabolized per liter of liver tissue per day) under the various bioassay conditions are listed in Table 2.

The MFO pathway saturates between 200 and 500 ppm of DCM in inhaled air. Consequently the predicted amounts of MFO metabolites formed in lung and liver tissue are nearly identical for the two dose levels in the inhalation study (2000 and 4000 ppm; Table 2). This prediction is inconsistent with the observation that liver and lung tumors are consistently higher in the 4000 ppm exposure group than the 2000 ppm exposure group in the inhalation study (Table 3).

The PB-PK model also predicts that the levels of MFO metabolites produced by administration of 250 mg/kg/day of DCM to $B_6C_3F_1$ mice in drinking water would be similar to the amounts of MFO metabolites produced in the inhalation study (Table 2). This prediction is also inconsistent with the hypothesis that MFO metabolites are responsible for the tumorigenicity of DCM, because no statistically significant increases in tumors were seen in the drinking water study (Table 1).

Predicted rates of metabolism of DCM by the GSH pathway correlate much better with the induction of lung and liver tumors in these two chronic studies. Predicted

Table 2. - *Predicted amounts of DCM metabolized by the oxidative (MFO) pathway or the conjugative pathway (GSH) in lung and liver tissue under bioassay conditions. Values given are mg equivalents of DCM metabolized by the indicated pathway per liter of tissue per day. Inhalation values are multiplied by 5/7 to correct for exposure 5 days/week*

	Control	Inhal (2000)	Inhal (4000)	Water (250)
LIVER				
MFO	0	3573	3701	5197
GSH	0	851	1811	15
LUNG				
MFO	0	1531	1583	1227
GSH	0	123	256	1

Table 3. - *Tumor incidences (combined adenomas and carcinomas) in male and female $B_6C_3F_1$ mice as reported by the National Toxicology Program [1] in a chronic inhalation study (6 h/day, 5 days/week) of DCM for chronic toxicity and/or carcinogenicity*

	ppm in chamber atmosphere		
	0	2000	4000
MALE MICE			
Liver tumors	44%	49%	67%
Lung tumors	10%	54%	80%
FEMALE MICE			
Liver tumors	6%	33%	83%
Lung tumors	6%	63%	85%

levels of GSH metabolites at 4000 ppm are higher than the predicted levels of GSH metabolites at 2000 ppm (Table 2). In contrast, the predicted levels of GSH metabolites formed in target tissues during administration of DCM in drinking water are very low. Thus the pattern of tumor induction in both studies shows a good correlation with the rates of metabolism of DCM by the GSH pathway.

Another indication that the GSH pathway plays a critical role in the induction of lung and liver tumors comes from long term inhalation bioassays of DCM in the Syrian Golden hamster. Burek et al. [8] reported that exposure of hamsters to DCM concentrations up to 3500 ppm failed to affect the incidences of tumors in the lung and liver (or any other site). The relative insensitivity of the hamster correlates well with *in vitro* measurements of the levels of MFO and GSH transferase in tissue preparations from the lung and livers of various species [9].

Homogenates of lung and liver from $B_6C_3F_1$ mice contained high levels of both MFO and GSH-transferase (Table 4). In contrast, tissue homogenates from the lung and livers of hamsters contained very low levels of GSH-transferase, although the levels of MFO activity in the livers of hamsters were slightly higher than in the livers of $B_6C_3F_1$ mice (Table 4). Again, the relative levels of GSH-transferase activity in the two species correlates well with the sensitivity of these species to the tumorigenicity of DCM, but the relative levels of MFO are inconsistent with the bioassay results. It is noteworthy that the levels of GSH-transferase in homogenates of human tissue are similar to those present in hamster tissues, but much lower than those in tissues of the mouse.

Having accepted the hypothesis that BRIs from the GSH pathway are involved in the tumorigenicity of DCM, it is possible to incorporate quantitative information from the PB–PK model into a quantitative risk assessment for DCM [9,10]. In the absence of a PB–PK model, Singh *et al.* [11] assumed that the amount of DCM-derived material (the "delivered dose") arriving at the target sites was directly proportional to the concentration of DCM in inhaled air from very high concentrations (4000 ppm) to very low concentrations (< 1 ppm) as shown by the dashed line in Fig. 2.

This assumption is a bad approximation of the dose-dependency of GSH metabolites in the target tissues. The solid line in Fig. 2 shows the dose-dependency of GSH metabolites predicted by the PB–PK model. Levels of GSH metabolites predicted by the PB–PK model are close to those obtained by linear extrapolation at concentrations above 1000 ppm. However, a marked nonlinearity in the curve is apparent in the region where MFO becomes saturated in mice (200-500 ppm). At low concentrations (1 ppm) where MFO is below saturation, the levels of GSH metabolite predicted by the PB–PK model are about 20 fold lower than predicted by linear extrapolation from 4000 ppm. This suggests that linear extrapolation of tumorigenicity results obtained at 4000 ppm down to low levels of DCM exposure (such as might be encountered by human populations) may significantly overestimate hazard.

Singh *et al.* [11] also employed another assumption to extrapolate between species (i.e. from $B_6C_3F_1$ mice to humans). They assumed that on a mg/kg/day basis, hu-

Table 4. - *Enzyme activities measured* in vitro *in tissue preparations from $B_6C_3F_1$ mice, F344 rats, Syrian Golden Hamsters, and healthy human accident victims. Details of the enzyme preparation and enzyme assays may be found in Reitz* et al. *[9]. MFO enzymes were assayed at a substrate concentration of 5 mM DCM, while GSH-transferase enzymes were assayed at a substrate concentration of 40 mM DCM. Activities are given in nmoles/min/mg protein*

	MFO		GSH	
	Liver	Lung	Liver	Lung
Mouse	11	5	26	7
Hamster	14	1	1	< 0.2
Rat	4	0.2	7	1
Human	5	< 0.1	2	0.4

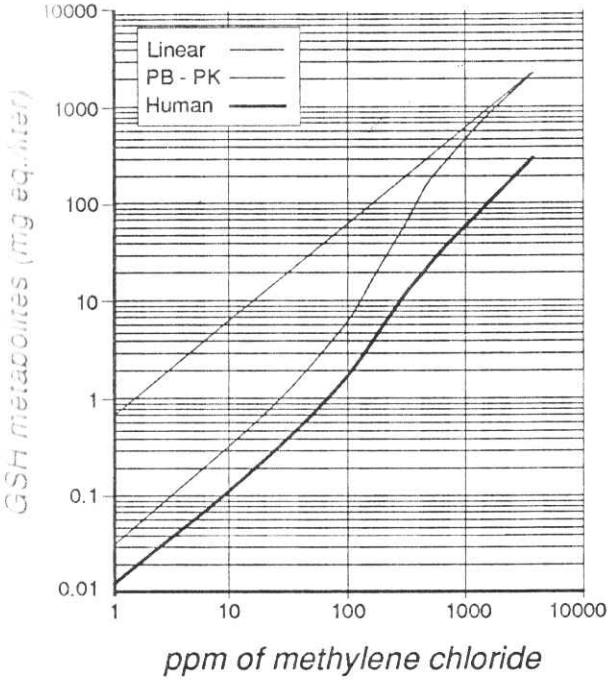

Fig. 2. - Dose-dependency of GHS metabolism.

mans were approximately 13 times more sensitive to DCM toxicity (including carcinogenicity) than mice (the "surface area rule"). This rule of thumb is probably approximately correct when parent chemical is directly responsible for toxicity, because the smaller species have higher levels of detoxifying enzymes and hence are better able to protect themselves by removing the chemical from the body. However, in the case of DCM, metabolism by the GSH pathway serves to activate DCM, so this assumption is inappropriate.

The heavy solid line in Fig. 2 shows the dose-dependency of GSH metabolism on inhaled concentration for humans as calculated by the PB–PK model. It is noteworthy that throughout the entire range of exposure concentrations, the PB–PK predicts that exposure of humans and $B_6C_3F_1$ mice to equivalent concentrations of DCM will result in lower values of the "delivered dose" for humans. Consequently, interspecies extrapolations of DCM tumorigenicity from $B_6C_3F_1$ mice to humans with the "surface area" rule will significantly overestimate the actual risk to humans.

The net effect of replacing the two default assumptions of Singh *et al.* [11] with quantitative information based on mechanistic information and PB–PK modeling has been summarized elsewhere [9]. Using the linearized multistage model of Howe and Crump [12], Singh *et al.* [11] calculated that the lifetime risk for humans continuously exposed to 1 microgram/cubic meter of DCM was 4.1×10^{-6} [11]. Incorporation of PB–PK principles into this same procedure reduced the calculated upper boundary on risk to 3.7×10^{-8} [9].

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