Chapter 8

Comparison of Cancer Risk Estimates for Vinyl Chloride Using Animal and Human Data with a PBPK Model

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Abstract

Vinyl Chloride (VC) is a trans-species carcinogen, producing tumors in a variety of tissues, from both inhalation and oral exposures, across a number of species. In particular, exposure to VC has been associated with a rare tumor, liver angiosarcoma, in a large number of studies in mice, rats, and humans. The mode of action for the carcinogenicity of VC appears to be a □henobarbi straightforward example of DNA adduct formation by a reactive metabolite, leading to mutation, mistranscription, and neoplasia. The objective of the present analysis was to investigate the comparative potency of a classic genotoxic carcinogen across species, by performing a quantitative comparison of the carcinogenic potency of VC using data from inhalation and oral rodent bioassays as well as from human epidemiological studies. A physiologically based pharmacokinetic (PBPK) model for VC was developed to support the target tissue dosimetry for the cancer risk assessment. Unlike previous models, the initial metabolism of VC was described as occurring via two saturable pathways, one representing low capacity-high affinity oxidation by CYP2E1 and the other (in the rodent) representing higher capacity-lower affinity oxidation by other isozymes of P450, producing in both cases chloroethylene oxide (CEO) and chloroacetaldehyde (CAA) as intermediate reactive products. Depletion of glutathione by reaction with CEO and CAA was also described. Animal-based risk estimates for human inhalation exposure to VC using total metabolism estimates from the PBPK model were consistent with risk estimates based on human epidemiological data, and were lower than those currently used in environmental decision-making by a factor of 80.

Introduction

Vinyl chloride (VC) has been produced commercially since the 1920s (Maltoni et al. 1981). It has been used as a refrigerant, an extraction solvent, an aerosol-propellant, and even as an ingredient in drug and cosmetic products. However, when it became evident that VC was carcinogenic both in animals and in humans, these uses were discontinued; the current use of VC is limited to serving as a chemical precursor in the production of such materials as polyvinyl chloride (PVC) and copolymer resins (USEPA 1985). Nevertheless, VC is also produced from the biodegradation of trichloroethylene by bacteria in the soil. Thus past spills of trichloroethylene may lead to current or future exposures of the public to VC in drinking water or other environmental media. The current potency estimates for VC published by the Environmental Protection Agency (EPA) do not quantitatively incorporate pharmacokinetic information on VC into the risk calculations (USEPA 1985). The purpose of the study reported here was to re-evaluate the quantitative risk to humans of cancer from exposure to VC, using pharmacokinetics to describe the dose-response for carcinogenicity in both animals and humans.

Evidence for the Carcinogenicity of VC

In 1967, Viola and co-workers initiated the first long-term bioassays using VC, in which male Wistar rats were exposed via inhalation (Viola et al. 1971). They reported an significant increase in the incidence of skin and lung carcinomas and osteochondromas. Since that time, the carcinogenicity of VC has been well established in several animal species by a number of routes of exposure. Frequently observed tumor sites in animals following VC exposure include the liver, kidney, lung, brain, mammary glands, and Zymbal glands. Of the many different tumor types which have been reported in animal bioassays of VC, three are of greater concern because they have been seen reproducibly at low concentrations (250 ppm and below): liver angiosarcoma, nephroblastoma, and mammary gland adenocarcinoma (Purchase et al. 1985). Hepatocellular carcinomas have also been occasionally observed at these low concentrations, particularly in studies of exposure to young animals (Bolt et al. 1980). Of these four low-concentration tumors, two are particularly notable in that they are rarely seen in unexposed animals: liver angiosarcoma and nephroblastoma.

In 1974, Creech and Johnson reported for the first time an association between exposure to VC and cancer in man: three cases of liver angiosarcoma were reported in men employed in a PVC plant (Maltoni et al. 1981). Angiosarcoma of the liver is considered to be a very rare type of cancer, with only 20-30 cases per year reported in the U.S. (Gehring et al. 1978, ATSDR 1993). Greater than expected incidences of angiosarcoma of the liver have since been reported in a number other cohorts of workers occupationally exposed to VC (Bryen et al. 1976, Fox and Collier 1977, Infante 1976, Jones et al. 1988, Monson et al. 1974, Pirastu et al. 1990, Rinsky et al. 1988, Teta et al. 1990, Waxweiler et al. 1976, Weber et al. 1981, Wong et al. 1986, Wu et al. 1989). Increased death due to cancer associated with VC exposure has also been reported for brain, lung, and hematopoietic systems (USEPA 1985), as well as for other tissues, but several analyses have concluded that liver angiosarcomas show the clearest evidence for causal association and also demonstrate the highest relative risk (Purchase et al. 1985). The correspondence across species for liver hemangiosarcoma is quite striking and has made this tumor the primary focus for VC risk assessments in recent years. In particular, the correspondence across species for this tumor suggests a unique opportunity for comparing quantitative cancer risk estimates based on epidemiological data with those derived from animal bioassay data.

Previous Risk Assessments for VC

The EPA has performed a series of risk assessments on VC as new information has become available regarding its carcinogenic potency (USEPA 1985). In 1980, a carcinogenic potency estimate for ingestion of contaminated drinking water was determined to be 1.74×10^{-2} $(mg/kg/day)^{-1}$, based on the incidence of total tumors among rats exposed by inhalation at 50 to 10,000 ppm, with a correction for the relationship between inhalation and oral dose levels and a cube-root body-weight-ratio correction to convert from animal to human

potency. This latter correction is commonly referred to as the body-surface-area (BSA) adjustment for cross-species extrapolation. In 1984, this oral potency was revised on the basis of an oral intubation study in rats; the revised oral potency was 1.4096×10^{-1} $(mg/kg/day)^{-1}$, based specifically on liver angiosarcoma rather than total tumors, and again using the BSA adjustment.

In 1985, data on the total incidence of lung and liver tumors in female rats treated with diets containing VC-fortified PVC (Feron et al., 1981) was used to calculate an oral potency of 1.9 $(mg/kg/day)^{-1}$, which yields a unit risk of 5.4×10^{-5} $(\mu g/L)^{-1}$. At the same time, an inhalation potency was calculated based on the studies of Maltoni et al. (1981, 1984) in which rats were exposed to concentrations of VC ranging from 1 to 30,000 ppm, 4 hrs/day, 5 days/wk, for 52 weeks. The resulting inhalation potency, based on liver angiosarcoma and applying the BSA adjustment, was 2.95×10^{-1} $(mg/kg/day)^{-1}$, yielding a unit risk of 8.4×10^{-5} $(\mu g/m^3)^{-1}$. The increase in the oral potency estimate from previous values was presumed to result from the availability of better tumor incidence data below the level of saturation of metabolism (USEPA 1985). It was also noted at that time that the oral potency, expressed in $(mg/kg/day)^{-1}$, was higher than the inhalation potency by roughly a factor of eight, suggesting that VC was more effective by the oral route. A subsequent study of the effect of exposure route on potency of carcinogens (Pepelko, 1991) also found that VC appeared to be more potent by the oral route when the comparison was made on the basis of mg/kg/day, and suggested that pharmacokinetic differences could underlie at least part of the discrepancy.

A pharmacokinetically-based risk assessment was proposed for VC as early as 1978 (Gehring et al., 1978). In this study it was demonstrated that the incidence of angiosarcoma correlated with amount of VC metabolized, which demonstrated Michaelis-Menten saturable kinetics, rather than with the exposure concentration of VC itself. Based on this observation and mechanistic arguments, it was proposed that daily amount metabolized should be used as the dose measure in a risk assessment for VC. Based on the limited pharmacokinetic and bioassay data available at that time, a pharmacokinetic human risk estimate for liver angiosarcoma was attempted. Their estimates of human risk at occupational exposure levels greatly overestimated the risks compared to estimates from epidemiological studies (Fox and Collier, 1977), while their predicted low-dose risks were many orders of magnitude lower than those estimated by the EPA. However, interpretation of this risk estimate is complicated by the authors' use of the probit (log probability) dose-response model rather than a linear model.

A more complete pharmacokinetic risk analysis for VC was performed (Chen and Blancato 1989) using a physiologically-based pharmacokinetic (PBPK) description of VC kinetics and total metabolism, patterned after the PBPK model for styrene (Ramsey and Andersen 1984). Metabolism of VC was modeled with a single saturable pathway, and the kinetic constants were estimated from measurements of whole body clearance (e.g. Filser and Bolt, 1979), although no attempt was made to validate the model against data on blood time-courses or

total metabolism in rodents or humans. The model was used to calculate total metabolism of VC (representing total production of reactive metabolites) as the dose metric in a carcinogenic risk assessment for liver tumors from exposure to VC. Human inhalation potency estimates based on the internal dose metric (mg VC metabolized/kg/day) were derived from the incidence of liver tumors in rat bioassays of VC performed by Maltoni et al. (1981, 1984).

The same PBPK model was then used to estimate total metabolism for oral bioassays of VC (Maltoni et al. 1981, 1984, Feron et al. 1981). Liver tumor incidence for the oral route predicted using the PBPK dose metric (mg metabolized/kg/day) to extrapolate from the inhalation potency agreed well with observed tumor incidence in the oral bioassays. This correspondence across routes based on metabolized dose contrasted with the apparently higher potency of VC by the oral route when the comparison was based on @inhaled@ versus Aingested@ dose in mg/kg/day.

Chen and Blancato (1989) also used the PBPK model to calculate the human inhalation potency for VC induced liver cancer based on epidemiological data (Fox and Collier, 1977). Using the same pharmacokinetic dose measure (mg metabolized/kg/day), the inhalation potency estimated from epidemiological data ($3.8x10^{-3}$/ppm) was essentially identical to the potency estimated from rat inhalation data (1.7 – $3.7x10^{-3}$/ppm), using body weight scaling (that is, without applying the BSA adjustment). However, a second, more complicated comparison performed in the same analysis, which involved time-to-tumor modeling, suggested that the BSA adjustment might be necessary to accurately predict lifetime-exposure human cancer fatality from animal incidence data.

More recently, a PBPK model of VC was developed by Reitz et al. (1996) and applied to compare cancer potency in mice, rats, and humans. The structure of the model was similar to that of Chen and Blancato (1989), providing a description of parent chemical kinetics and total metabolism based on the styrene model of Ramsey and Andersen (1984). Metabolism of VC was modeled with a single saturable pathway, and the kinetic constants were estimated from fitting of closed chamber gas uptake studies with rats. The model was then validated against data on total metabolism in the rat (Watanabe et al., 1976b), gas uptake data in the mouse, and inhalation data in the human (Baretta et al., 1969). The model was used to calculate total metabolism of VC as the dose metric in a carcinogenic risk assessment. Based on the rat inhalation bioassay of Maltoni et al. (1981, 1984), and using the linearized multistage model, they estimated that lifetime continuous human exposure to 1.75 $\mu g/m^3$ VC is associated with an increased lifetime risk of one in a million. This estimate equates to a lifetime risk of approximately 1.5×10^{-3}/ppm, in good agreement with the results of Chen and Blancato (1989). The potency estimates from rats were then shown to be consistent with tumor incidence data in mice and humans when the pharmacokinetic dose metric was used.

Pharmacokinetics and Metabolism of VC

Numerous studies on the pharmacokinetics and metabolism of VC have been conducted, with the majority of these studies conducted in rats (Withey 1976, Hefner et al. 1975, Guengerich and Watanabe 1979, Bolt et al. 1976, 1977, Watanabe et al. 1976a, 1976b, 1978, Jedrychowski et al. 1984, 1985, Tarkowski et al. 1980). A simplified diagram of the metabolism of VC is shown in Figure 1. The primary route of metabolism of VC is by the action of the mixed function oxidase (MFO) system, now referred to as Cytochrome P450 or CYP, on VC to form chloroethylene oxide (Bolt et al. 1977, Plugge and Safe 1977). Chloroethylene oxide (CEO) is a highly reactive, short-lived epoxide that rapidly rearranges to form chloroacetaldehyde (CAA), a reactive □-halocarbonyl compound; this conversion can also be catalyzed by epoxide hydrolase (Pessayre et al. 1979).

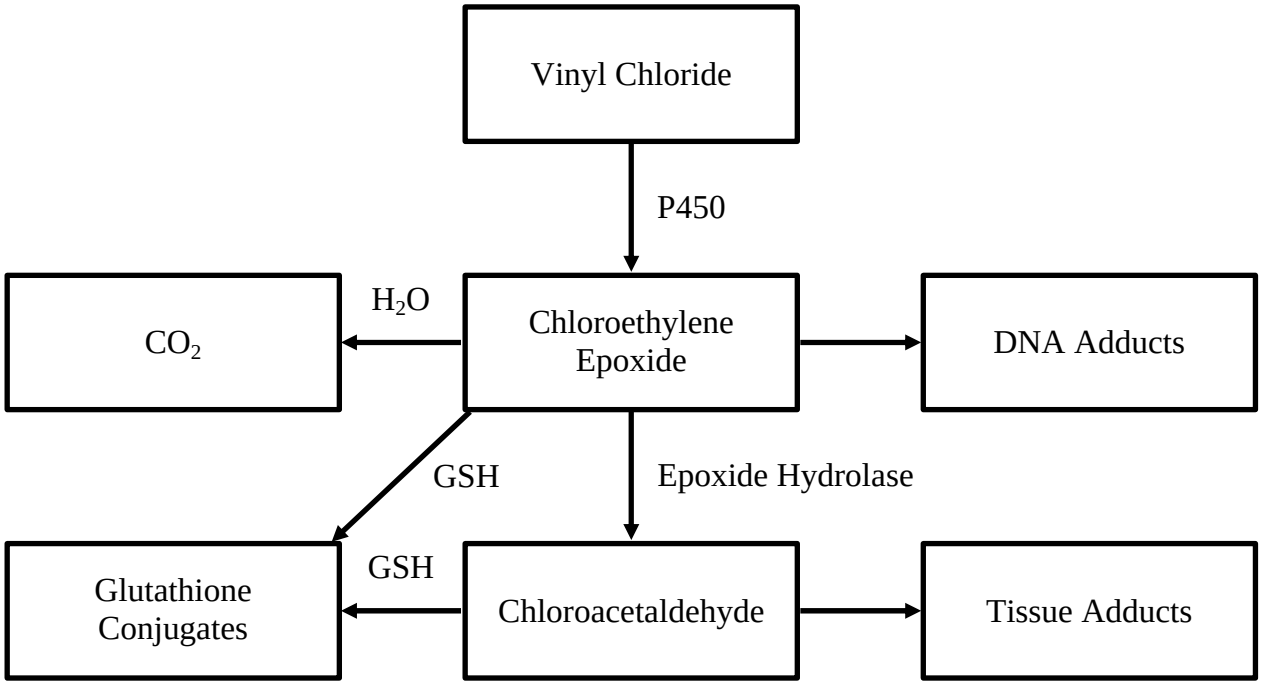


Figure 1. Diagram of the metabolism of vinyl chloride (VC). Abbreviations: P450 – Cytochrome P450 (CYP); GSH – glutathione.

The main detoxification of these two metabolites is conjugation binding with glutathione (Jedrychowski et al. 1985, Leibman 1977, Tarkowski et al. 1980). This hypothesis is supported by the observation of decreased non-protein sulfhydryl concentrations at high VC exposure concentrations (Jedrychowski et al. 1985, Tarkowski et al. 1980), as well as by the excretion of glutathione (GSH) conjugated metabolites in the urine, observed in rats following exposure to VC (Watanabe et al. 1976c, Hefner et al. 1975). CAA may also combine directly or enzymatically via glutathione transferase (GST) with GSH to form S-formylmethylglutathione. S-Formylmethylglutathione, through direct interaction with GSH derived cysteine, can be excreted as N-acetyl-S-(2-hydroxyethyl)cysteine, another major urinary metabolite of VC (Green and

Hathway 1975). The GSH conjugates are then subject to hydrolysis resulting in excretion of cysteine conjugates in the urine (Hefner et al. 1975). Two of the three major urinary metabolites of VC in rats have been identified as N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid (Watanabe et al. 1976b).

Based on the elimination of VC observed following administration by various routes of exposure, the metabolism of VC appears to be a dose-dependent, saturable process (Green and Hathway 1975, Bolt 1978, Hefner et al. 1975, Gehring et al. 1977, 1978). Following exposure via oral or inhalation routes to low doses of VC, metabolites are excreted primarily in the urine. However, once the saturation point for metabolism is reached, VC is eliminated via other routes, primarily exhalation of the parent compound (Gehring et al. 1977, Watanabe et al. 1976b, Watanabe and Gehring 1976). The route of elimination of VC also depends on the route of administration, with urinary excretion favored more following oral or intraperitoneal administration, indicating a first-pass effect due to liver metabolism (Bolt 1978).

The specific isozymes of the P450 system involved in the metabolism of VC have not yet been unequivocally established. However, it is clear from both *in vitro* and *in vivo* studies that several isozymes can play a role. High affinity, low capacity oxidation by CYP 2E1 is probably responsible for essentially all of the metabolism of VC at low concentrations in uninduced animals and humans (Guengerich et al. 1991). There is also evidence for a significant increase in metabolism in animals pretreated with □henobarbital (Ivanetich et al. 1977), suggesting that CYP 2B1 also metabolizes VC. At high concentrations *in vivo*, the metabolism of VC in rats leads to a destruction of P450 enzyme (Reynolds et al. 1975), which is greatly enhanced in □henobarbital- or Arochlor- induced animals (Arochlor induces CYP 1A2). The loss of P450 has been suggested to result from the production of reactive intermediates during the metabolism of VC (Guengerich and Strickland 1977), and is inhibited by GSH *in vitro* (Ivanetich et al. 1977). Induction of P450 by □henobarbital or Arochlor was also necessary to produce acute hepatotoxicity from VC in rats (Jaeger et al. 1977).

The contribution of several P450 isozymes to the metabolism of the related compound trichloroethylene has been studied in the male Wistar rat and male B6C3F1 mouse (Nakajima et al. 1993). Using monoclonal antibodies specific to each isozyme, the investigators were able to determine that: (1) CYP2E1 contributes more to the metabolism of TCE in mice than in rats, (2) CYP2C11/6 contributes more to the metabolism of TCE in rats than in mice (CYP2C11/6 is a constitutive, non-inducible isozyme present only in male rodents), (3) CYP1A1/2 contributes to the uninduced metabolism of TCE in mice but not in rats, and (4) CYP2B1 does not contribute to the metabolism of TCE in naive animals of either species.

Summarizing the above observations, it appears that at low concentrations the metabolism of VC is primarily due to CYP2E1, but that at higher concentrations, where CYP2E1 becomes capacity-limited, other isozymes may contribute to its metabolism. The extent of this higher-capacity metabolism is

likely to vary across animal species, strain, and sex. To the extent that such higher capacity, lower affinity metabolism (referred to in future as "non-2E1" metabolism) may be important in conducting a risk assessment for VC, it will have to be characterized separately for each species, strain, and sex of interest. From a pharmacokinetic modeling perspective, non-2E1 metabolism would be handled as a second saturable metabolic pathway with a larger value for the Michaelis-Menten constant (KM). For example, it has been demonstrated that the metabolism of the related compound, vinyl bromide, is best described with two distinct saturable pathways having different affinities (Gargas and Andersen, 1982). Of major importance for human risk assessment, some of the low-affinity, high capacity constitutive (2C11/6) and inducible (2B1/2) P450 isozymes in the rodent may have no human correspondents (Guengerich, 1987).

Pharmacokinetic Modeling of VC

The pharmacokinetic models that have previously been used in risk assessments for VC (Gehring et al. 1978, Chen and Blancato, 1989, Reitz et al. 1996) have in common the assumption of a single saturable pathway for the metabolism of VC. However, another published model of VC (Gargas et al., 1990) differed from the models discussed above by the incorporation of a second, linear metabolic pathway (presumed by the authors to be GSH conjugation) in parallel with the saturable (oxidative) pathway. Based on gas uptake studies in the male F344 rat, both a saturable and a linear metabolic component were postulated for VC.

These alternative descriptions of metabolism in the published models of VC were examined in a more in-depth study of VC pharmacokinetics performed for the U.S. Air Force by several of the present authors (Clement, 1990). The one- and two-pathway descriptions were refit to gas uptake data and then compared with measurements of total metabolism by Gehring et al. (1978) and Watanabe et al. (1976b). Although the two-pathway description provided a significantly better fit to the gas uptake data (adding parameters nearly always improves a fit), the resulting parameters tended to overpredict total metabolism at higher concentrations owing to the presence of the first-order component. In addition, it was not possible to explain the continued increase in glutathione (GSH) depletion measured at the highest exposure levels (where the saturable component was above saturation) because only products of the oxidative metabolism of VC have been shown to react with GSH. In an attempt to provide a better correspondence to the data on both total metabolism and glutathione depletion, two possible refinements to the model were investigated. In the first, direct reaction of VC with GSH was postulated, and in the second, the products of both the saturable and the linear pathways were assumed to react with GSH. Unfortunately, neither description was able to provide a satisfactory correspondence to both total metabolism and GSH depletion data. The analysis suggested that a different formulation featuring two saturable oxidative pathways, both producing reactive metabolites, might provide the required behavior.

The PBPK models previously used to conduct a human risk assessment for VC (Chen and Blancato, 1989, Reitz et al. 1996) certainly provide a more biologically plausible basis for estimating human carcinogenic risk than default measures of VC exposure. However, a 2-saturable pathway model structure would have the potential advantage of being able to reproduce experimental data on both total metabolism and GSH depletion, based on a reasonable hypothesis: only saturable oxidative metabolism is involved (no other metabolic pathway for VC has been demonstrated), and only products of oxidative metabolism react with GSH (neither direct reaction nor GST-mediated conjugation of VC with GSH has been demonstrated). This metabolic hypothesis formed the basis for the model development conducted in this study. The model, which will be described in more detail later, is similar to the PBPK model developed by D'Souza and Andersen (1988) to describe vinylidine chloride (VDC) kinetics and toxicity, including the depletion of GSH by the products of VDC metabolism. The chief difference is the use of two saturable pathways instead of one to describe metabolism. The use of a low affinity pathway in parallel with the high affinity pathway is necessary to provide the continued increases in total metabolism and GSH depletion observed with VC in rats.

Mechanism of Carcinogenicity of VC

Many of the results of the pharmacokinetic and metabolism studies discussed above indicate that like other chlorinated alkenes, VC must be metabolized to cause carcinogenicity (Bartsch and Montesano 1975). A reactive, short-lived metabolite, which achieves only low steady-state concentrations, is thought to be responsible for the toxic effects of VC (Bolt 1978). That the toxicity of VC is mediated by the production of reactive metabolites is suggested by the results of *in vitro* studies in which enhanced mutagenicity was observed if microsomal enzymes of fortified liver homogenates were present (Bartsch et al. 1975, Malavielle et al. 1975, Rannug et al. 1974). The rapid elimination of VC and its major metabolites is also consistent with the theory that a shortly lived, reactive metabolite occurring at concentrations too low for direct observation could be responsible for the carcinogenicity of VC (Bolt et al. 1977).

Both CEO and CAA have been evaluated as possible carcinogenic metabolites of VC, because both compounds can react with tissue nucleophiles (Guengerich et al. 1981). However, comparative studies have shown that CEO is carcinogenic in skin, and acts as an initiator in the initiation/promotion protocol, but that CAA does not (Zajdela et al. 1980). Moreover, CEO has been found to display 400-fold greater mutagenic potency than CAA in bacterial mutagenicity assays (Perrard 1985). Comparative experiments have also been conducted with VC and 2,2'-dichlorodiethylether which support the conclusion that CEO is the carcinogenic metabolite (Bolt 1986, Gwinner et al. 1983). These experiments made use of an important difference between the metabolism of 2,2'-dichloroethylether and VC: only the metabolism of VC leads to an intermediary epoxide. While both 2,2'-Dichloroethylether and VC are metabolic precursors of CAA, only VC is a precursor of CEO. After administration of 2,2'-dichloroethylether to rats, protein alkylation in the liver was comparable to that

observed with VC; however, no adduct formation was observed. Preneoplastic hepatocellular ATP-deficient foci were also reported in rats following exposure to VC, but not 2,2'-dichlorodiethylether (Gwinner et al. 1983). These results support the conclusion that CEO is the ultimate carcinogenic metabolite of VC.

The metabolism of VC to products irreversibly bound to DNA and protein was examined *in vitro* with rat liver microsomes to investigate the possible role of CAA in the carcinogenicity of VC. Inhibition studies were performed with alcohol dehydrogenase, which is the enzyme involved in the breakdown of CAA, and epoxide hydrolase, which is the enzyme involved in the breakdown of CEO. Alcohol dehydrogenase was effective in inhibiting the binding of VC metabolites to protein, while epoxide hydrolase was effective in inhibiting the binding of VC metabolites to DNA. These results also support the conclusion that the epoxide is the carcinogenic moiety (Guengerich et al., 1981). More recent studies have also demonstrated that all of the major identified DNA adducts formed from VC exposure are derived solely from CEO (Laib 1986, Guengerich 1992). One possible explanation for the ability of CEO, rather than CAA, to form adducts with DNA is that CEO is lipophilic, while CAA is hydrophilic; this difference could impact the compound's relative abilities to cross the nuclear membrane (Bolt et al. 1980).

Adduct Formation from VC

It has long been a tenet of carcinogenic risk assessment that the mechanism of carcinogenicity for "genotoxic" carcinogens (sometimes referred to as initiators) involves reaction with DNA, leading to mistranscription during subsequent cell division, causing a loss or change in heritable information which results in a neoplastic daughter cell (Van Duuren 1988, Singer 1985). As early as 1978, it was demonstrated that binding of VC to liver macromolecules following inhalation exposure of rats correlated well with both total metabolism and the observed incidence of angiosarcoma (Watanabe et al. 1978). It was suggested that the carcinogenicity of VC was due to binding with DNA and subsequent miscoding during cell reproduction, although no DNA or RNA binding was actually observed in that study. Subsequently, a DNA adduct produced by VC exposure was identified: 7-(2-oxoethyl)guanine (OEG); however, although this adduct represents roughly 98% of all VC adducts (Swenberg et al. 1992), it is very rapidly repaired and does not appear to lead to miscoding during DNA replication. Therefore it is not considered important for carcinogenesis (Laib 1986, Swenberg et al. 1992).

The *in vivo* formation of four etheno-DNA adducts have since been demonstrated following exposure of animals to VC (Laib 1986, Fedtke et al. 1990, Dosanjh et al. 1994): 1,N^2-ethenoguanine (1,N^2-EG); N^2,3-ethenoguanine (N^2,3-EG); 1,N^6-etheno-2'-deoxyadenosine (EDA), and 3,N^4-etheno-2'-deoxycytidine (EDC). These etheno-adducts, although produced at much lower concentrations than OEG, are highly persistent (Swenberg et al. 1992) and can lead to defective transcription (Singer et al. 1987); for example, EG produces a base pair mismatch (G->A transition) in bacterial assays (Cheng et al. 1990). Single-

strand breaks (SSBs) have been detected in liver DNA following inhalation exposure of mice to VC (Walles et al. 1988). (It is generally assumed that SSBs represent an intermediate stage in the excision repair of DNA adducts.) The occurrence of SSBs reached a maximum at exposures of 500 ppm, consistent with saturation of metabolism. It was found that 20% of the SSBs remained after 20 hours.

It is still not possible to determine which, if any, of the DNA-adducts identified from VC exposure may be responsible for the observed carcinogenicity of VC. The likelihood that a given DNA-adduct will lead to a neoplastic transformation depends on many factors, including its persistence as well as the consequences of its repair or failure to be repaired. The persistence of a given adduct depends on both the rate of formation and the rate of repair (Singer 1985); in humans, all of the etheno adducts appear to be repaired by the same DNA glycosylase, but not at the same rate (Dosanjh et al. 1994). In particular, the repair of the ethenoguanines (1,N^2-EG and N^2,3-EG) appears to be much slower than that of the other etheno-adducts (EDA and EDC) in humans (Dosanjh et al. 1994). This is in contrast to the results of a similar study in rats, where N^2,3-EG was repaired with a half-life of about 30 days, while there was no evidence that EDA and EDC were repaired at all (Swenberg et al. 1992).

In summary, although there is strong circumstantial evidence linking etheno-DNA adducts with the observed carcinogenicity of VC, there is not yet sufficient information to provide a quantitative link between the tissue concentrations of a specific adduct and the risk of cancer in that tissue. The ratio of the concentrations of the various etheno adducts in the rat is only marginally consistent across tissues and studies (Fedtke et al. 1990, Swenberg et al. 1992), and there are no data on relative adduct levels in VC exposed humans. Amount of metabolism would still appear to provide the best dose metric for comparison with tumor incidence.

Other Considerations in the Carcinogenicity of VC

The majority of the DNA adduct studies conducted with VC have been conducted on or related to the hepatocyte. However, although VC is primarily metabolized in the hepatocyte (Ottenwalder and Bolt 1980), the primary target cell for carcinogenicity in the liver is the sinusoidal cell, as indicated by the incidence of liver angiosarcoma in both animals and humans. Sinusoidal cells show a relatively low activity for transforming VC into reactive, alkylating metabolites, roughly 12% of the activity of hepatocytes (Ottenwalder and Bolt 1980). Therefore, it has been suggested that the carcinogenic metabolites of VC may have to migrate from the hepatocytes to produce tumors in the sinusoidal cells (Laib and Bolt 1980). This possibility was suggested by Laib and Bolt (1980) following their observation that alkylating metabolites of VC were capable of diffusing through an artificial semipermeable membrane in a model *in vitro* system. In studies conducted *in vitro* with rat hepatocytes by Guengerich et al. (1981), more than 90% of the hexane-insoluble metabolites were found to migrate out of the cell, with more than 70% of the total irreversibly bound

species found outside the cell. These results were interpreted to indicate that the majority of the reactive metabolites can leave the intact hepatocyte. On the other hand, sinusoidal cells do possess the ability to produce reactive metabolites from VC, albeit at a slower rate than the hepatocyte (Ottenwalder and Bolt 1980). In either case, the greater susceptibility of the sinusoidal cells to the carcinogenic effects of VC may result from an inability of the sinusoidal cells to repair one or more of the DNA adducts produced by VC as efficiently as the hepatocytes.

Another concern with regard to the carcinogenicity of VC is the relative sensitivity of young animals. Newborn rats treated with VC respond with both angiosarcoma and hepatocellular carcinoma, in contrast with adult animals, which generally evidence only angiosarcoma (Maltoni et al. 1981). Consistent with this observation, VC was found to induce preneoplastic foci in newborn rats, but not in adults (Laib et al. 1979). Interestingly, in the same study it was found that VC did induce preneoplastic foci in adult rats after partial hepatectomy, indicating that the appearance of foci, and presumably of hepatocellular carcinoma, in neonatal animals was a consequence of the increased rate of cell proliferation at that age. Support for this hypothesis was obtained in studies with 11-day old Wistar rats (Laib et al. 1989). Following inhalation exposure to VC, roughly 5-fold higher levels of OEG were found in the livers of the 11-days old rats as compared to similarly treated adult rats. It was also determined that the rate of cell proliferation in the livers of the 11-day old rats was roughly 8-fold higher than in the adults. These results are consistent with the suggestion that the apparent sensitivity of young animals to VC-induced liver cancer is related to enhanced DNA alkylation and by increased cellular proliferation at an early age (Laib et al. 1989). In a similar study, roughly 4-fold greater concentrations of both OEG and EG were also seen in preweanling rats exposed to VC (Fedtke et al. 1990).

This apparent increased sensitivity of newborn animals occurs in spite of a much lower metabolic capability at birth: during the first week of life, the P450 activity in the liver of rats increases from about 4% to about 80% of adult levels (Filser and Bolt 1979). However, the low metabolic capability at birth probably explains why VC is not a transplacental carcinogen (Bolt et al. 1980). The potential implications of the higher cell proliferation rates found in newborn animals provide sufficient cause for extra prudence regarding exposure of infants to VC or any other DNA-reactive carcinogen. Nevertheless, cell proliferation and DNA adduct data alone is not adequate to demonstrate a quantitative differential between acceptable adult and infant exposures. In comparative studies of partial lifetime exposure of rats to VC (Drew et al. 1983), exposure from 0 to 6 months showed an overall similar potency to exposure from 6 to 12 months of life. In particular, for exposure to VC from 0 to 6 months the incidence of hepatocellular carcinoma and hemangiosarcoma was 4.0% and 5.3%, respectively, while for exposure from 6 to 12 months, the incidence was 11.5% and 3.8%. Thus this comparative bioassay does not provide support for an increased sensitivity due to early age exposure for hepatocellular carcinomas specifically, or for VC-induced carcinogenicity in general.

Selection of a Risk Assessment Approach

Based on the information described above on the pharmacokinetics, metabolism, and mechanism of carcinogenicity of VC, it is necessary to determine the appropriate approach for conducting a human risk assessment. Clearly, the evidence is strong that the carcinogenicity of VC is related to the production of reactive metabolic intermediates. The most appropriate pharmacokinetic dose metric for a reactive metabolite is the total amount of the metabolite generated divided by the volume of the tissue into which it is produced (Andersen et al. 1987a). In the case of VC, reasonable dose metrics for angiosarcoma would include the total amount of metabolism divided by the volume of the liver (RISK), or the total amount of metabolism not detoxified by reaction with glutathione, again divided by the volume of the liver (RISKM). A third, less likely possibility, that the GSH conjugate of VC is subsequently metabolized to a reactive species which is responsible for the carcinogenicity, can also be considered by using a dose metric based on the total amount of reaction with GSH divided by the volume of the liver (RISKG). The assumption underlying the use of these dose metrics is that the concentration of the actual carcinogenic moiety, or the extent of the crucial event associated with the cellular transformation, is linearly related to this pseudo-concentration of reactive intermediates, and that the relationship of the actual carcinogenic moiety or crucial event to the dose metric is constant across concentration and species. Specifically, the average amount generated in a single day is used, averaged over the lifetime (i.e., the lifetime average daily dose, or LADD). The use of a dose rate, such as the LADD, rather than total lifetime dose, has been found empirically to provide a better cross-species extrapolation of chemical carcinogenic potency (USEPA, 1992).

Subsequent steps in the carcinogenic mechanism related to specific adduct formation, detection, and repair, as well as to the consequences of DNA mistranscription and the potential impact of increased cell proliferation, have only been sketchily outlined and have not yet reached the point where they could be incorporated into a risk assessment in any quantitative form. However, there appears to be sufficient evidence to justify the assumption that VC acts as a classic initiator, producing genetic transformations through direct reaction of its metabolites with DNA. Therefore the traditional assumption of low-dose linearity of risk appears to be warranted, and the linearized multistage (LMS) model would seem to be the most appropriate for low-dose extrapolation.

Description of PBPK Model for VC

The PBPK model for VC developed in this study is shown in Figure 2. As mentioned earlier, the model is basically an adaptation of a previously developed PBPK model for vinylidene chloride (D'Souza and Andersen 1988). For a poorly soluble, volatile chemical like VC, only four tissue compartments are required: a richly perfused tissue compartment which includes all of the organs except the liver, a slowly perfused tissue compartment which includes all of the muscle and skin tissue, a fat compartment which includes all of the fatty tissues, and a liver

compartment. All metabolism is assumed to occur in the liver, which is a good assumption in terms of the overall kinetics of VC, but which would have to be revised to include target-tissue-specific metabolism if a serious attempt were to be made to perform a VC risk assessment for a tissue other than the liver (Andersen et al. 1987a). The model also assumes flow-limited kinetics, or venous equilibration; that is, that the transport of VC between blood and tissues is fast enough for steady state to be reached within the time it is transported through the tissues in the blood.

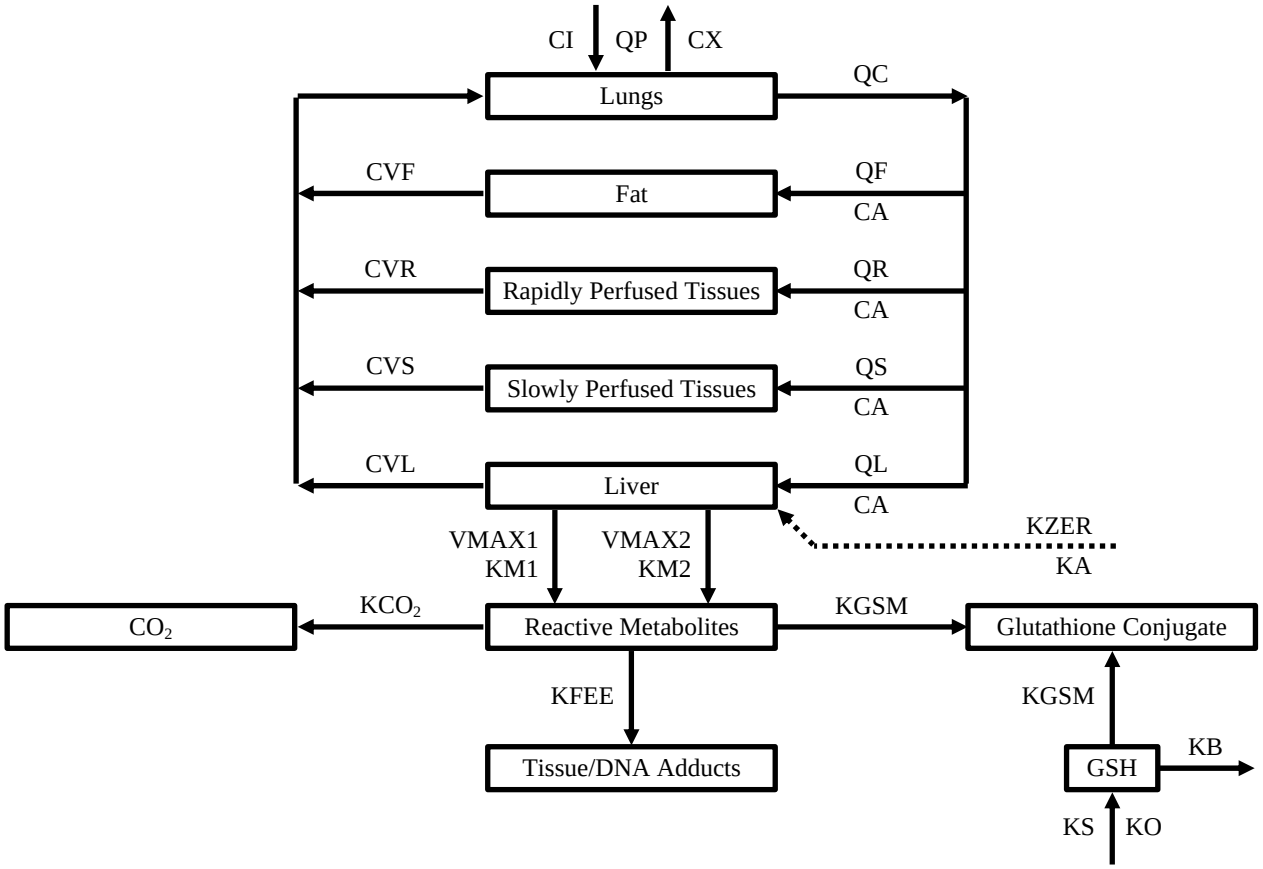


Figure 2. Diagram of the PBPK model of VC. Abbreviations: QP – alveolar ventilation; CI – inhaled concentration; CX – exhaled concentration; QC – cardiac output; QF, CVF – blood flow to, and venous concentration leaving, the fat; QR, CVR – blood flow to, and venous concentration leaving, the richly perfused tissues (most organs); QS, CVS – blood flow to, and venous concentration leaving, the slowly perfused tissues (e.g., muscle); QL, CVL – blood flow to, and venous concentration leaving, the liver; VMAX1,KM1 – capacity and affinity for the high affinity oxidative pathway enzyme (CYP 2E1); VMAX2,KM2 – capacity and affinity for the lower affinity oxidative pathway enzymes, (e.g., CYP 2C11/6); KZER – zero-order rate constant for uptake of VC from drinking water; KA – first-order rate constant for uptake of VC from corn oil; KCO2 – first-order rate constant for metabolism of VC to CO_2; KGSM – first-order rate constant for reaction of VC metabolites with GSH; KFEE – first-order rate constant for reaction of VC metabolites with other cellular materials, including DNA; KB – first-order rate constant for normal turnover of GSH; KO – zero-order rate

constant for maximum production of GSH; KS – parameter controlling rate of recovery of GSH from depletion.

Metabolism of VC is modeled by two saturable pathways, one high affinity, low capacity (with parameters VMAX1C and KM1) and one low affinity, high capacity (with parameters VMAX2C and KM2). Subsequent metabolism is based on the metabolic scheme shown in Figure 1: the reactive metabolites (whether CEO, CAA, or other intermediates) may then either be metabolized further, leading to CO_2, react with GSH, or react with other cellular materials, including DNA. Because exposure to VC has been shown to deplete circulating levels of GSH, a simple description of GSH kinetics was also included in the model.

The parameters for the model are listed in Tables 1 and 2. The physiological parameters are EPA reference values (USEPA 1988), except for the alveolar ventilation (QPC) in the human, which was calculated from the standard EPA value for the ventilation rate in the human, 20 m^3/day, assuming a 33% pulmonary dead-space (USEPA 1988). The value for the cardiac output (QCC) in the human was selected to correspond to the same workload as the standard EPA ventilation using data from Astrand and Rodahl (1970). For modeling of the closed chamber studies with human subjects, more typical resting values of 15 for cardiac output (QCC) and and 18 for alveolar ventilation (QPC) were used. In some cases, it was also necessary to slightly vary the alveolar ventilation and cardiac output of the animals in the closed chamber studies in order to obtain acceptable simulations of those experiments. The partition coefficients for Fisher-344 (F344) rats were taken from Gargas et al. (1989), and those for Sprague Dawley rats were taken from Barton et al. (1995). The Sprague Dawley values were also used for modeling of Wistar rats. Blood/air partition coefficients for the other species were obtained from Gargas et al. (1989), and the corresponding tissue/blood partition coefficients were estimated by dividing the Sprague Dawley rat tissue/air partition coefficients by the appropriate blood/air value.

Table 1
Model Parameters and Dose Metrics for the Vinyl Chloride Model

		Mouse (CV-%)[1]	Rat (CV-%)	Human (CV-%)
BW	Body Weight (kg)	--[2] (11)	-- (11)	70.0 (30)
QPC	Alveolar Ventilation (L/hr, 1 kg animal)	30.0 (58)	21.0 (58)	24.0 (16)
QCC	Cardiac Output (L/hr, 1 kg animal)	18.0 (9)	18.0 (9)	16.5 (9)
Tissue Blood Flows (Fraction of Cardiac Output):				
QRC	Rapidly Perfused Tissues	0.51 (50)	0.51 (50)	0.5 (20)
QFC	Fat	0.09 (60)	0.09 (60)	0.05 (30)
QSC	Slowly Perfused Tissues	0.15 (40)	0.15 (40)	0.19 (15)
QLC	Liver	0.25 (96)	0.25 (96)	0.26 (35)
Tissue Volumes (Fraction of Body Weight):				
VSC	Slowly Perfused Tissues	0.77 (30)	0.75 (30)	0.63 (30)
VFC	Fat	-- (30)	-- (30)	0.19 (30)
VRC	Richly Perfused Tissues	0.035 (30)	0.05 (30)	0.064 (10)
VLC	Liver	0.055 (6)	0.04 (6)	0.026 (5)
Partition Coefficients:				
PB	Blood/Air	2.26 (15)	2.4 (15)	1.16 (10)
PF	Fat/Blood	10.62 (30)	10.0 (30)	20.7 (30)
PS	Slowly Perfused/Blood	0.42 (20)	0.4 (20)	0.83 (20)
PR	Richly Perfused/Blood	0.74 (20)	0.7 (20)	1.45 (20)
PL	Liver/Blood	0.74 (20)	0.7 (20)	1.45 (20)

[1] CV-%: Coefficient of Variation = 100 * Standard Deviation / Mean
[2] See Table 2

Table 1 (cont.)
Model Parameters And Dose Metrics For The Vinyl Chloride Model

		Mouse (CV-%)	Rat (CV-%)	Human (CV-%)
Metabolic Parameters:				
VMAX1C	Maximum Velocity of First Saturable Pathway (mg/hr, 1 kg animal)	-- (20)	-- (20)	4.0 (30)
KM1	Affinity of First Saturable Pathway (mg/L)	0.1 (30)	0.1 (30)	1.0 (50)
VMAX2C	Maximum Velocity of Second Saturable Pathway (mg/hr, 1 kg animal)	-- (20)	-- (20)	0.1 (0)
KM2	Affinity of Second Saturable Pathway (mg/L)	10.0 (30)	10.0 (30)	10.0 (50)
GSH Parameters:				
KCO2C	First Order CEO Breakdown to CO_2	1.6 (20)	1.6 (20)	1.6 (20)
KGSMC	Conjugated Rate Constant with Metabolite	0.13 (20)	0.13 (20)	0.13 (20)
KFEEC	Conjugated Rate Constant with Non-GSH	35.0 (20)	35.0 (20)	35.0 (20)
GSO	Initial GSH Concentration	5800.0 (20)	5800.0 (20)	5800.0 (20)
KBC	First Order Rate Constant for GSH Breakdown	0.12 (20)	0.12 (20)	0.12 (20)
KS	Constant Controlling Resynthesis	2000.0 (20)	2000.0 (20)	2000.0 (20)
KOC	Zero Order Production of GSH	28.5 (20)	28.5 (20)	28.5 (20)
Dosing Parameters:				
KA	Oral Uptake Rate (/hr)	3.0 (50)	3.0 (50)	3.0 (50)

Table 2
Strain/Study-Specific Parameter Values

		BW	VFC	VMAX1C	VMAX2C
Swiss Albino Mice (Inhalation Study)	Male	0.044	0.13	8.0	0.1[12]
	Female	0.040	0.12	5.0	3.0
Sprague-Dawley Rats (Inhalation Study)	Male – Low Dose	0.638	0.19	4.0	2.0
	Male – High Dose	0.433	0.13	4.0	2.0
	Female – Low Dose	0.485	0.20	3.0	0.1[1]
	Female – High Dose	0.321	0.14	3.0	0.1[1]
Sprague-Dawley Rats (Gavage Study)	Male – Low Dose	0.632	0.19	4.0	2.0
	Male – High Dose	0.405	0.12	4.0	2.0
	Female – Low Dose	0.445	0.18	3.0	0.1[1]
	Female – High Dose	0.301	0.13	3.0	0.1[1]
Wistar Rats (Dietary Study)	Male	0.436	0.14	4.0	2.0
	Female	0.245	0.11	3.0	0.1[1]

The affinity for the 2E1 pathway (KM1) in the rat, mouse, and hamster was set to 0.1 on the basis of studies of the competitive interactions between CYP2E1 substrates in the rat (Barton et al. 1995, Andersen et al. 1987b). The affinity used for the non-2E1 pathway (KM2) in the mouse and rat was set during the iterative fitting of the rat total metabolism, glutathione depletion, and rate of metabolism data, described below. The capacity parameters for the two oxidative pathways (VMAX1C and VMAX2C) in the mouse, rat, and hamster were estimated by fitting the model to data from closed-chamber exposures with each of the species and strains of interest (Barton et al. 1995, Bolt et al. 1977, Clement 1990, Gargas et al. 1990), holding all of the other model parameters fixed and requiring a single pair of values for VMAX1C and VMAX2C to be used for all of the data on a given sex/strain/species. Figures 3 and 4 show examples of the results of this fitting process for the male B6C3F1 mouse and male Wistar rat, respectively.

[12] The value of this parameter was normally set to zero. It was only set to 0.1 for the PBPK_SIM runs. The variance for this parameter was set to zero in the PBPK_SIM runs.

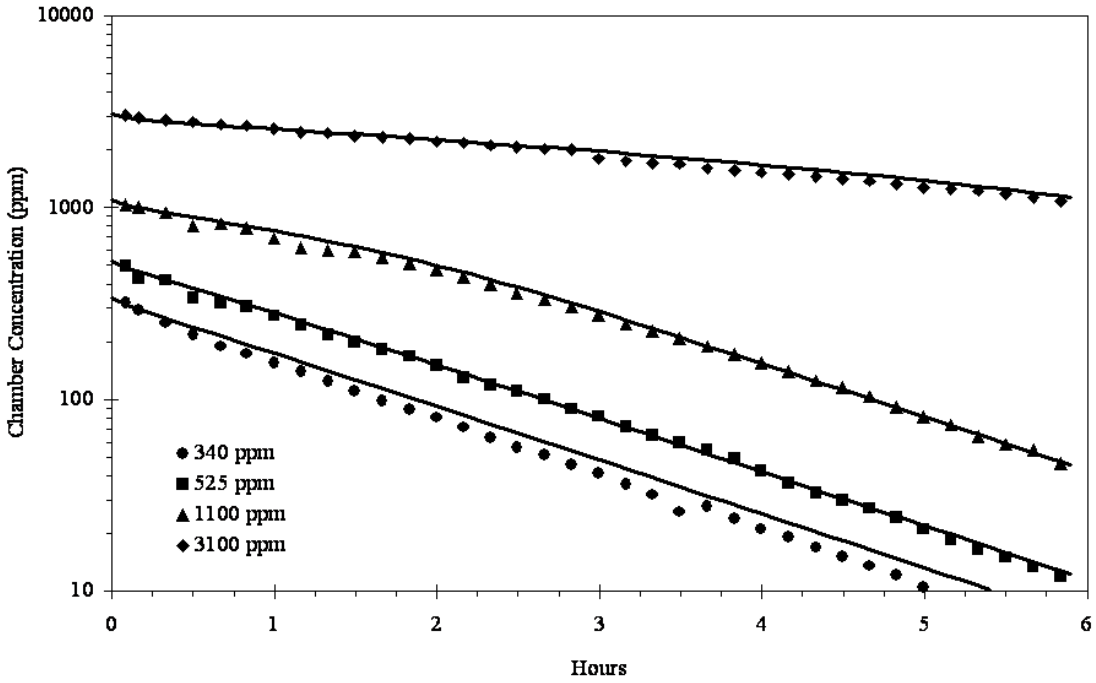


Figure 3. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of male B6C3F1 mice to VC in a closed, recirculated chamber (Data taken from Clement 1990).

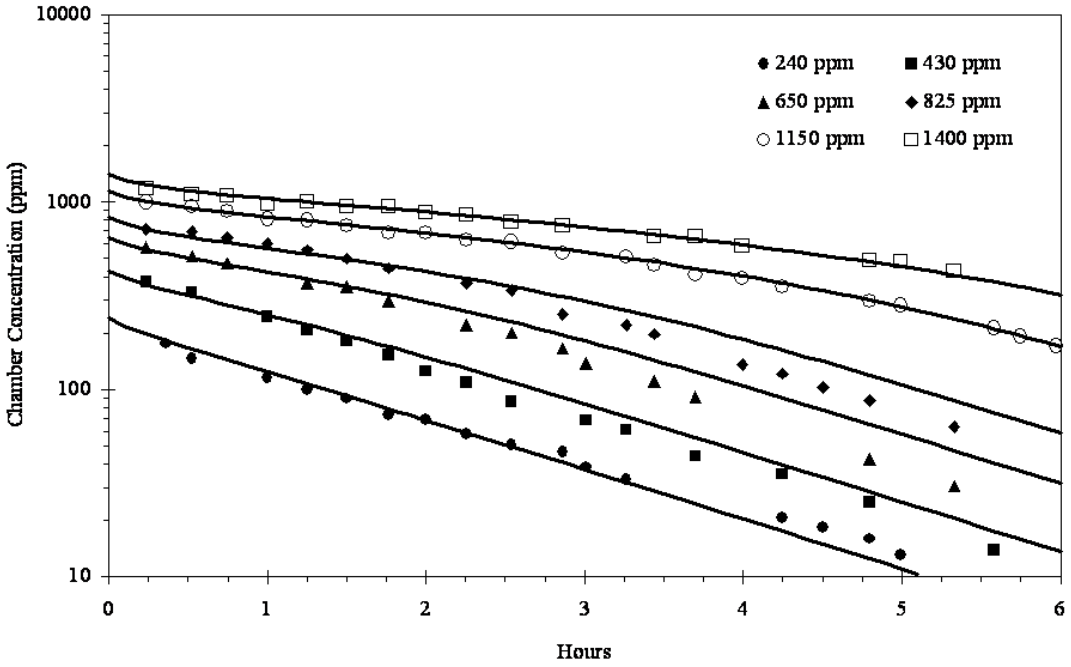


Figure 4. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of male Wistar rats to VC in a closed, recirculated chamber (Data taken from Bolt et al. 1977).

Initial estimates for the subsequent metabolism of the reactive metabolites and for the glutathione submodel in the rat were taken from the model for vinylidene chloride (D'Souza and Andersen, 1988). These parameter estimates, along with the estimates for VMAX2C and KM2, were then refined for the case of VC in the Sprague Dawley rat using an iterative fitting process which included the closed chamber data for the Sprague Dawley and Wistar rat (Barton et al. 1995, Bolt et al. 1977, Clement 1990) along with data on glutathione depletion (Jedrichowski et al. 1985, Watanabe et al., 1976c), total metabolism (Gehring et al. 1978), and CO_2 elimination (Watanabe and Gehring 1976). Since this last data set was obtained for oral dosing with VC in corn oil, a value for KA, the oral uptake rate from corn oil, was estimated from fitting of separate data on blood concentrations following dosing of rats with VC in corn oil (Withey 1976). The results of this iterative process are illustrated in Figures 4 through 8, and the resulting parameters are listed in Tables 1 and 2. The parameters obtained for the rat were used for the other species with appropriate allometric scaling (e.g., body weight to the -1/4 for the first order rate constants).

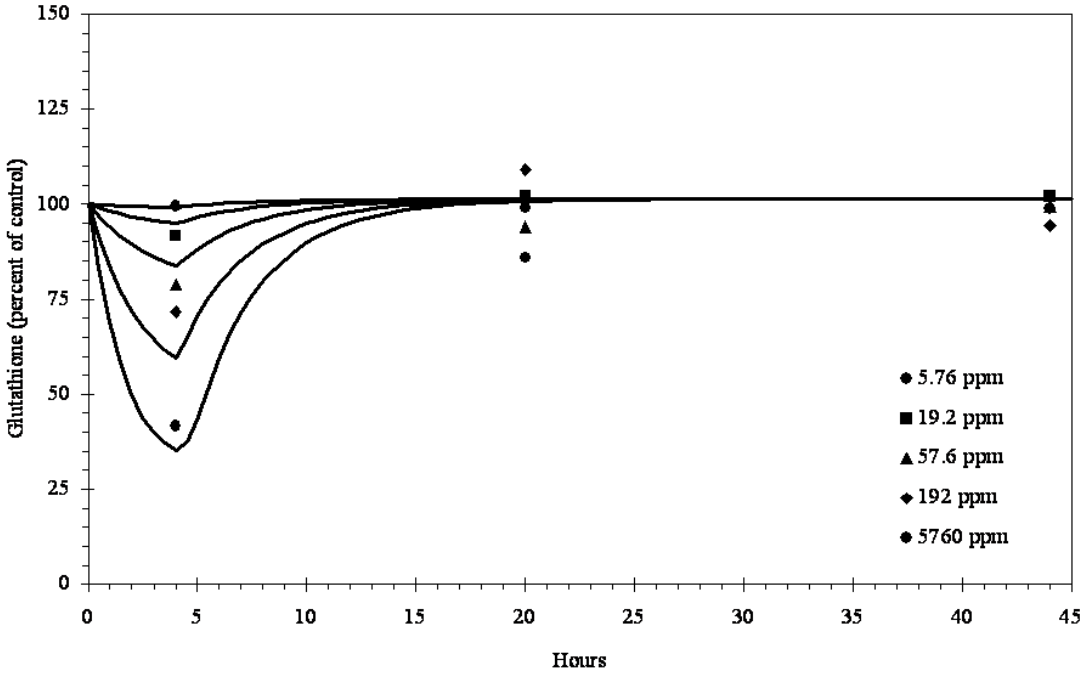


Figure 5. Model-predicted (lines) and experimentally determined (symbols) glutathione concentrations (as percent of control animal levels) 0, 16, and 40 hours following 4-hr inhalation exposures to VC at concentrations of (top to bottom) 15, 50, 150, 500, and 15000 mg/m^3 (Data taken from Jedrichowski et al. 1985).

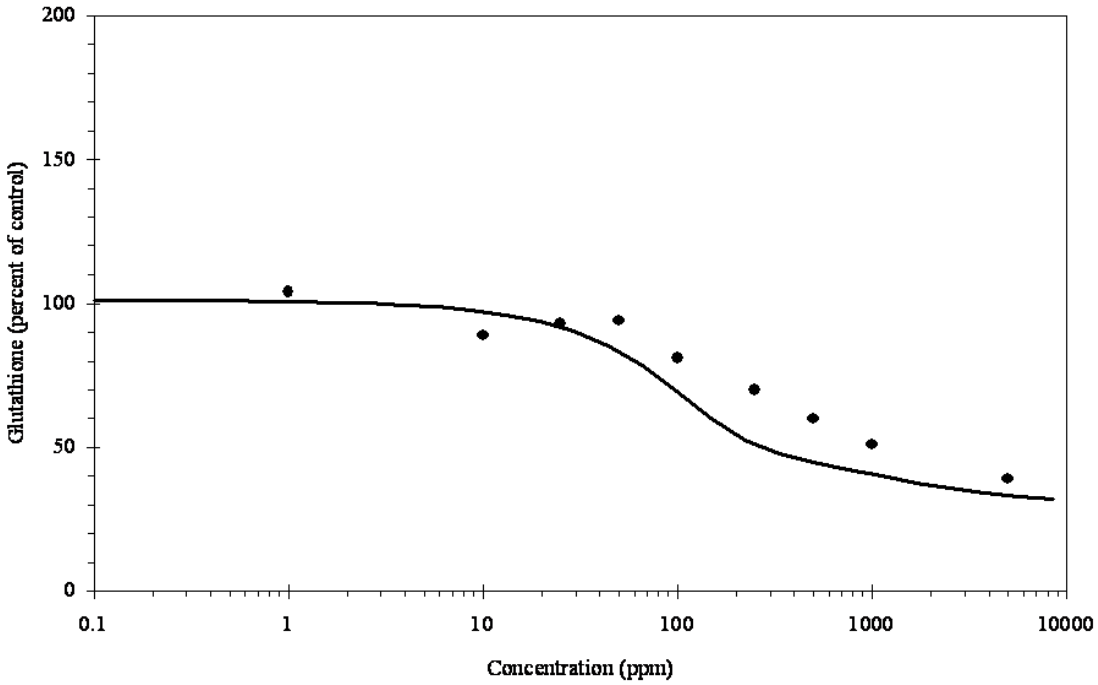


Figure 6. Model-predicted (lines) and experimentally determined (symbols) glutathione concentrations (as percent of control animal levels) immediately following 6-hr inhalation exposures to VC (Data taken from Watanabe et al. 1976c).

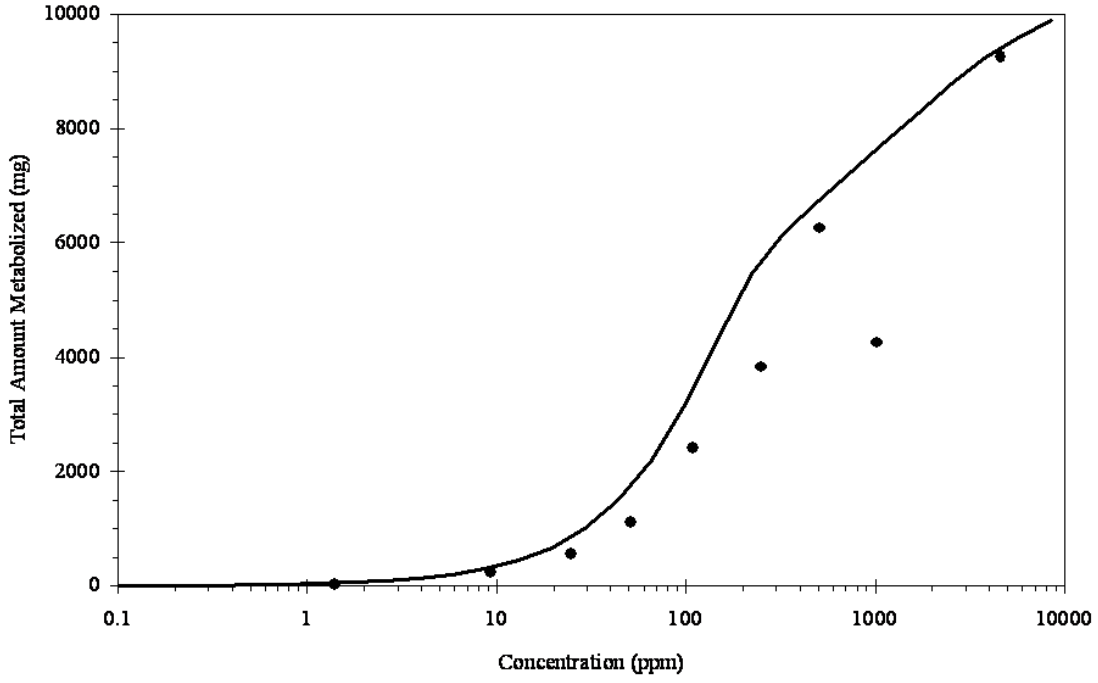


Figure 7. Model-predicted (lines) and experimentally determined (symbols) total amount metabolized during 6-hr inhalation exposures to VC (Data taken from Gehring et al. 1978).

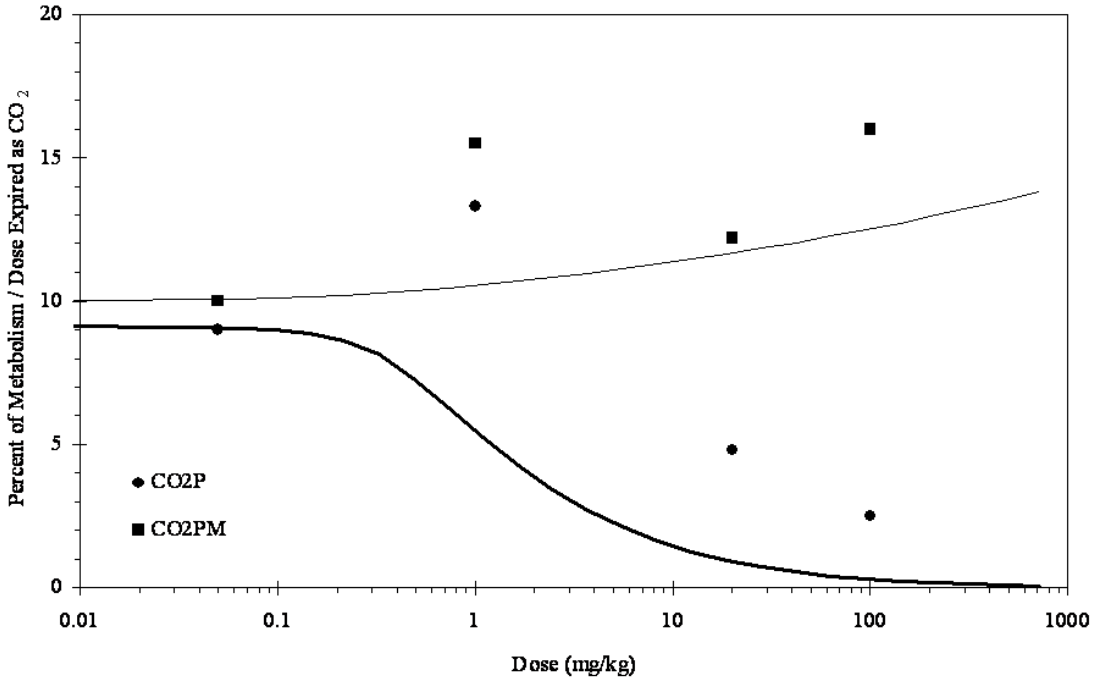
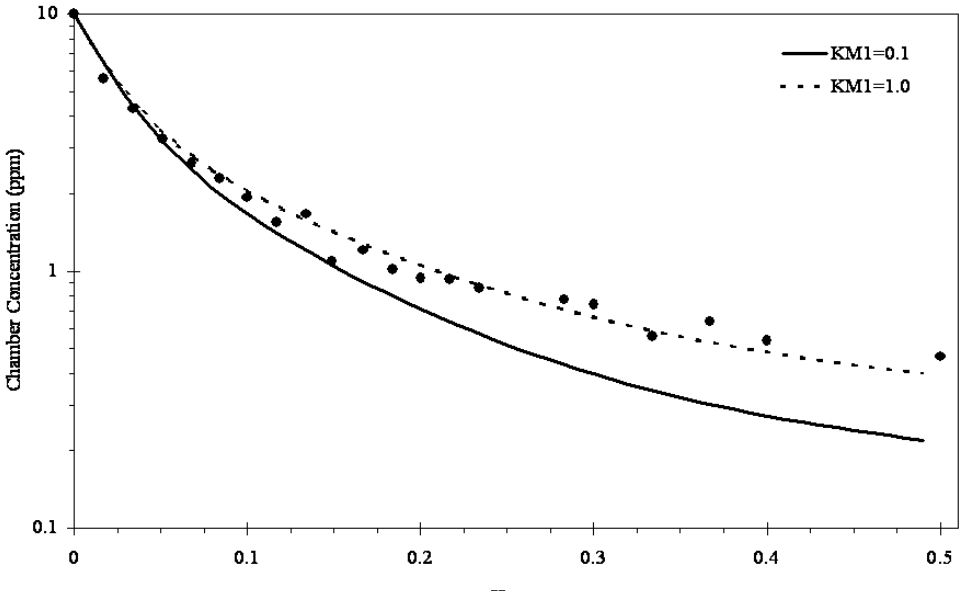


Figure 8. Model-predicted (lines) and experimentally determined (symbols) total expired CO_2, as a percent of total metabolism (upper line and symbols) and as a percent of dose (lower line and symbols), following oral dosing with VC in corn oil (Data taken from Watanabe and Gehring 1976).

Parameterization of the P450 metabolism pathways in the human was accomplished as follows: There is no evidence of high capacity, low affinity P450 metabolism for chlorinated ethylenes in the human; therefore, VMAX2C in the human was set to zero. The ratio of VMAX1C to KM1 could be estimated by fitting the model to data from closed chamber studies with two human subjects (Buchter et al. 1978), in a manner entirely analogous to the method used for the animal closed chamber analysis. The result of fitting the data on one of the two subjects is shown by the upper curve in Figure 9a, and the model prediction using the value estimated from this subject is compared with the data from the second subject in Figure 9b. The precision of the estimate of VMAX1C/KM1 can be evaluated by a comparison of the two model runs shown in these figures, for KM1=1.0 and KM1=0.1. It can be seen that the ratio of VMAX1C/KM1 varies between the two subjects. This variability of CYP2E1 activity in the human is not surprising; several studies have demonstrated a variability of human CYP2E1 activity of roughly an order of magnitude (Reitz et al. 1989, Sabadie et al. 1980). Much lower variability is observed in the inbred strains typically used in animal studies; for example, the coefficient of variation (standard deviation divided by the mean) for CYP2E1 activity in rats in one study of rodents was only 14% (Sabadie et al. 1980). The wide variability in human CYP2E1 activity is an important consideration for estimating the potential difference between average population risk and individual risk in a human cancer risk assessment for materials like VC, whose carcinogenicity depends on metabolic activation.

(a)



(b)

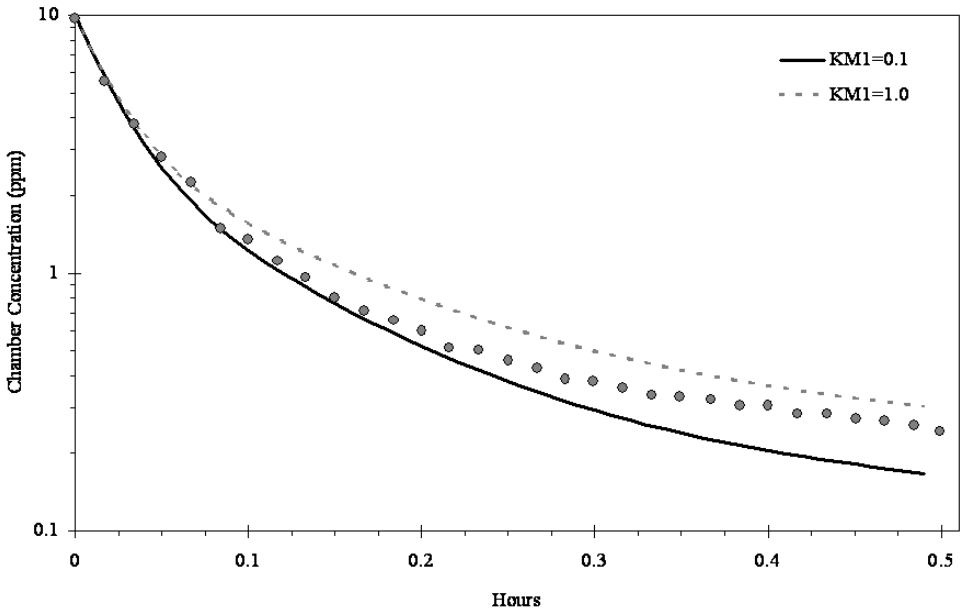
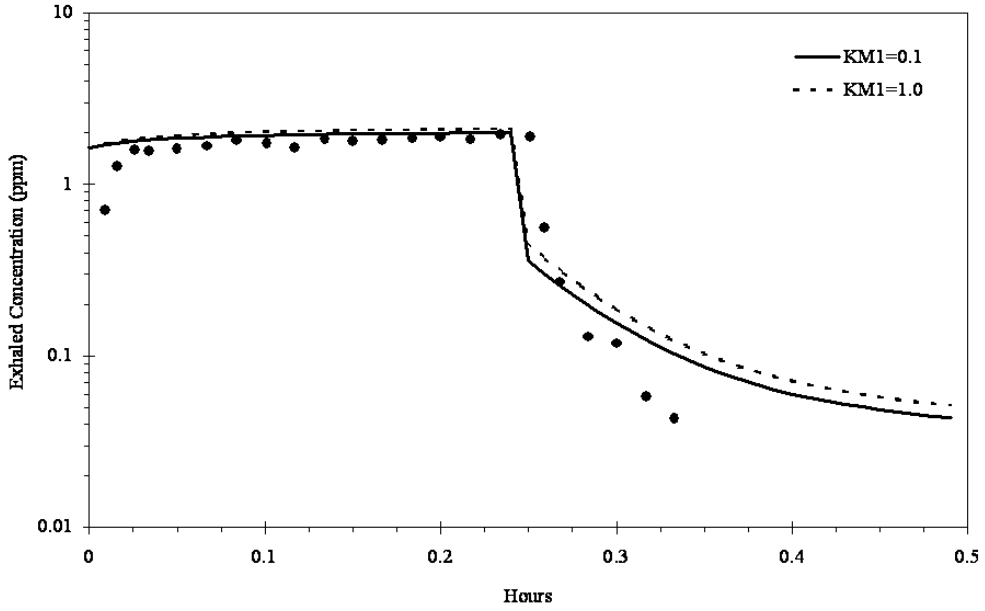


Figure 9. Model predictions (lines) and experimental data (symbols) for the chamber concentration during exposure of human subjects to VC in a closed, recirculated chamber (Data taken from Buchter et al. 1978). (a) Subject A – the lines show the model predictions for (top to bottom) KM1 = 1.0 and 0.1; (b) Subject B – the lines show the model predictions for (top to bottom) KM1 = 1.0 and 0.1. The rest of the model parameters are those shown for the human in Tables 3 and 4.

(a)



(b)

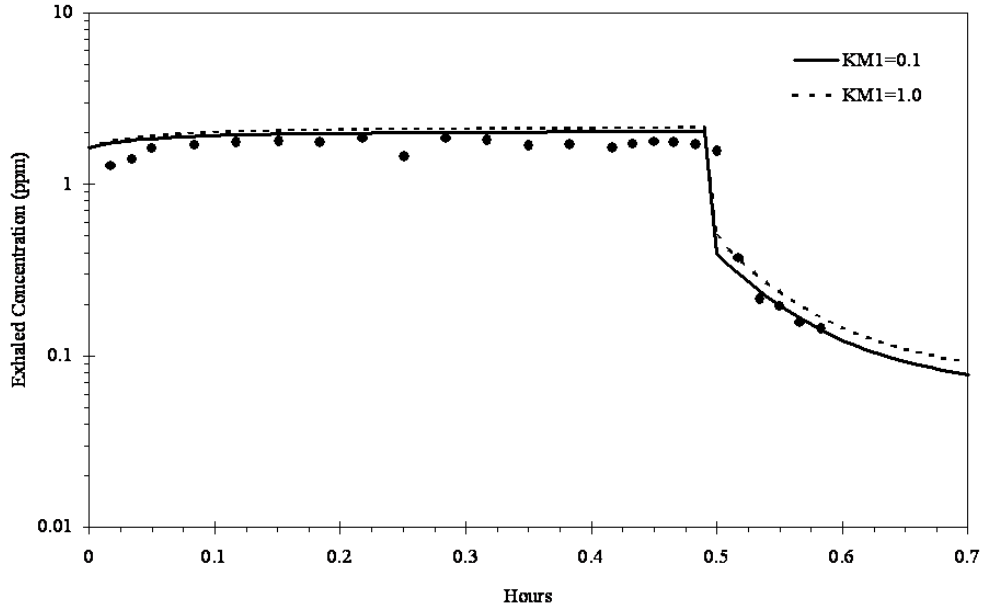


Figure 10. Model predictions (lines) and experimental data (symbols) for the exhaled air concentration during and following inhalation exposure of human subjects to a constant concentration of 2.5 ppm VC (Data taken from Buchter et al. 1978). (a) Subject A – the lines show the model predictions for (top to bottom) KM1 = 1.0 and 0.1; (b) Subject B – the lines show the model

predictions for (top to bottom) KM1 = 1.0 and 0.1. The rest of the model parameters are those shown for the human in Tables 3 and 4.

In order to obtain separate estimates of VMAX1C and KM1 in the human, higher exposure concentration closer to metabolic saturation would be required. Fortunately, cross-species scaling of CYP2E1 between rodents and humans appears to follow allometric expectations for metabolism very closely; that is, the metabolic capacity scales approximately according to body weight raised to the 3/4 power (Andersen et al. 1987a). Support for the application of this principal to VC can be obtained from data on the metabolism of VC in non-human primates (Buchter et al. 1980). Based on data for the dose-dependent metabolic elimination of VC in the rhesus monkey, the maximum capacity for metabolism can be estimated to be about 50 μmol/hr/kg. This equates to a VMAX1C (the allometrically scaled constant used in the model) of approximately 4 mg/hr for a 1 kg animal, a value which is in the same range as those estimated for the rodents from the closed chamber exposure data. The similarity of VMAX1C in humans and rats is also supported by an *in vitro* study which found the activity of human microsomes to be 84% of the activity of rat microsomes. Based on these comparisons, the human VMAX1C was set to the primate value and KM1 was calculated using this value of VMAX1C and the ratio of VMAX1C/KM1 obtained from the closed chamber analysis. The ability of the resulting human model to reproduce constant concentration inhalation exposure data (Buchter et al. 1978) is shown in Figures 10a and 10b. From a comparison of the model predictions for KM1 = 1.0 and KM1= 0.1, it can be seen that the reproduction of parent chemical concentrations in a constant concentration inhalation exposure is not a particularly useful test of the accuracy of the metabolism parameters in a PBPK model of a volatile compound. The discrepancies or agreement between the model and the data are primarily due to details of the physiological description of the individual, such as fat content, ventilation rate, blood/air partition, etc., rather than rate of metabolism.

Comparison of Risk Estimates for Human VC Inhalation

The model just described was used to calculate each of the pharmacokinetic dose metrics for angiosarcoma (RISK, RISKM, and RISKG) in the most informative of the animal bioassays (Maltoni et al. 1981, 1984, 1988, Feron et al. 1981), as well as for human inhalation exposure. The results of these calculations for the RISK dose metric (mg metabolized per kg liver) are shown in Table 3. The 95% upper confidence limits (UCLs) on the human risk estimates for lifetime exposure to 1 ppm VC were then calculated on the basis of each of the sets of animal bioassay data, using the LMS model. As shown in Table 4, the resulting risk estimates for lifetime human exposure to 1 ppm VC, based on the RISK dose metric, range from 1.52×10^{-3} to 15.7×10^{-3}. Because saturation of metabolism occurs well above the 1 ppm concentration in the human, estimates of risk below 1 ppm can be adequately estimated by assuming linearity (e.g., the risk estimates for lifetime human exposure to 1 ppb of VC would range from approximately 1.52×10^{-6} to 15.7×10^{-6}). It should be noted that although the animal studies represent both inhalation and oral exposure, the risk predictions in each case are for human inhalation exposure.

Table 3
Dose Metric Values for Angiosarcomas

					Daily Dose Metric	Lifetime Average Daily Dose
Study	Species	Duration	Dose	Incidence	RISK (mg/L)	RISK (mg/L)
Inhalation	Human	Continuous Exposure	1 ppm		1.614	1.614
Drinking Water			0.0286 mg/kg/d		0.603	0.603
Maltoni et al. 1988 (BT4) B Inhalation	Swiss Albino Mice (M)	4 hr/d, 5 d/wk for 30 of 104 wks	0 ppm	0/80		
			50 ppm	1/30	161.924	33.363
			250 ppm	9/30	775.615	159.811
			500 ppm	6/30	1245.220	256.570
			2500 ppm	6/29	1434.800	295.632
			6000 ppm	2/30	1479.270	304.795
			10000 ppm	1/26	1505.580	310.216
	Swiss Albino Mice (F)	4 hr/d, 5 d/wk for 30 of 104 wks	0 ppm	0/70		
			50 ppm	0/30	156.907	32.330
			250 ppm	9/30	673.015	138.671
			500 ppm	8/30	887.253	182.813
			2500 ppm	10/30	1197.670	246.773
			6000 ppm	11/30	1341.160	276.338
			10000 ppm	9/30	1405.330	289.560
Maltoni et al. 1981, 1984 (BT15 and BT1) - Inhalation	Sprague-Dawley Rats (M)	4 hrs/d, 5 d/wk for 52 of 147 wks	0 ppm	0/73		
			1 ppm	0/55	2.398	0.606
			5 ppm	0/47	11.985	3.028
			10 ppm	1/46	23.933	6.047
			25 ppm	4/40	59.552	15.047
		4 hrs/d, 5 d/wk for 52 of 135	50 ppm	1/29	117.989	32.463
			250 ppm	2/26	473.425	130.254

					Daily Dose Metric	Lifetime Average Daily Dose
Study	Species	Duration	Dose	Incidence	RISK (mg/L)	RISK (mg/L)
		wks	500 ppm	6/28	593.928	163.409
			2500 ppm	7/24	803.198	220.986
			6000 ppm	10/25	911.248	250.714
	Sprague-Dawley Rats (F)	4 hrs/d, 5 d/wk for 52 of 147 wks	0 ppm	0/45		
			1 ppm	0/48	2.343	0.592
			5 ppm	0/43	11.698	2.956
			10 ppm	0/42	23.332	5.895
			25 ppm	1/41	57.838	14.614
		4 hrs/d, 5 d/wk for 52 of 135 wks	50 ppm	0/26	113.653	31.270
			250 ppm	1/28	375.989	103.447
			500 ppm	0/22	425.029	116.939
			2500 ppm	6/26	488.374	134.367
			6000 ppm	3/17	522.359	143.718
Maltoni et al. 1981, 1984 (BT11) - Gavage	Sprague-Dawley Rats (M)	5 d/wk for 52 of 136 wks	0 mg/kg/d	0/60		
			0.021 mg/kg/d	0/15	0.488	0.133
			0.214 mg/kg/d	0/15	4.962	1.355
			0.714 mg/kg/d	1/21	16.373	4.472
			2.38 mg/kg/d	0/34	50.390	13.762
			11.9 mg/kg/d	4/39	133.231	36.387
			35.7 mg/kg/d	8/36	203.079	55.463
	Sprague-Dawley Rats (F)		0 mg/kg/d	0/73		
			0.021 mg/kg/d	0/18	0.477	0.130
			0.214 mg/kg/d	1/19	4.835	1.321
			0.714 mg/kg/d	2/29	15.800	4.315

					Daily Dose Metric	Lifetime Average Daily Dose
Study	Species	Duration	Dose	Incidence	RISK (mg/L)	RISK (mg/L)
			2.38 mg/kg/d	0/37	45.330	12.380
			11.9 mg/kg/d	6/34	102.763	28.066
			35.7 mg/kg/d	9/35	143.866	39.291
Feron et al. 1981 - Diet	Wistar Rats (M)	135 wks 7 d/wk	0 mg/kg/d	0/55		
			1.7 mg/kg/d	0/58	39.539	39.539
			5.0 mg/kg/d	6/56	116.103	116.103
			14.1 mg/kg/d	27/59	325.845	325.845
	Wistar Rats (F)	144 wks 7 d/wk	0 mg/kg/d	0/57		
			1.7 mg/kg/d	0/58	38.611	38.611
			5.0 mg/kg/d	2/59	113.243	113.243
			14.1 mg/kg/d	9/57	316.628	316.628

Table 4
Risk Estimates Based on Angiosarcoma Incidence

95% UCL Risk / 1000 / ppm	RISK
Maltoni et al. (1988) BT4-Inhalation	
- male mice	1.52
- female mice	3.27
Maltoni et al. (1981, 1984) BT14/BT1-Inhalation	
- male rats	5.17
- female rats	2.24
Maltoni et al. (1981, 1984) BT11-Gavage	
- male rats	8.68
- female rats	15.70
Feron et al. (1981) –Diet	
- male rats	3.05
- female rats	1.10

Only the results for the RISK dose metric (mg metabolized per kg liver) are shown in Tables 3 and 4. The p-values for goodness of fit of the 1-stage model using the other alternative dose metrics (RISKM, and RISKG) were very similar; therefore, it is not possible to select one metric over another on the basis of agreement with the dose-response of the incidence data. Fortunately, the risks predicted for each of the studies by the various dose metrics were roughly similar. The RISKM metric, which is the most biologically plausible, resulted in lower risks than the RISK metric (ranging from $0.50x10^{-3}$ to $12.46x10^{-3}$ /ppm); while the RISKG metric, which is probably the least likely, resulted in risk estimates up to 30 percent higher than those shown in Table 4.

There are no consistent differences between risk estimates based on male and female animals, with the female-based risks being higher than the male-based risks in some studies, and lower in others, but generally agreeing within a factor of two to three. The risk estimates based on inhalation studies with mice ($0.5x10^{-3}$ to $4.3x10^{-3}$/ppm) agree very well with those based on inhalation studies with rats ($1.46x10^{-3}$ to $5.94x10^{-3}$/ppm) , demonstrating the ability of pharmacokinetics to integrate dose-response information across species.

The risks estimated from the dietary administration of VC ($0.94x10^{-3}$ to $3.13x10^{-3}$/ppm) are also in good agreement with those obtained from the inhalation bioassays, showing good route-to-route correspondence of potency based on the pharmacokinetic dose metric. However, the estimates based on oral gavage of VC in vegetable oil ($6.58x10^{-3}$ to $16.3x10^{-3}$/ppm) are about 6-fold higher than either dietary or inhalation exposure. It has previously been noted in studies with chloroform that administration of the chemical in corn oil results in more marked hepatotoxic effects than observed when the same chemical is provided in an aqueous suspension (Bull et al. 1986). It has also been demonstrated that administration of corn oil alone leads to an increase in peroxisomal oxidative enzyme activity in rats (DeAngelo et al. 1989). The toxicity and oxidative environment created in the liver by continual dosing with large volumes of vegetable oil could serve to potentiate the effects of genotoxic carcinogens in the liver. In support of this suggestion, Newberne et al. (1979) found that incorporation of corn oil into the diet increased the yield of aflatoxin B_1-induced tumors in rats. A similar phenomenon could be responsible for the apparently higher potency of VC when administered by oil gavage compared to incorporation in the diet.

Epidemiological Analysis of Vinyl Chloride Carcinogenicity

In order to evaluate the plausibility of the risks predicted on the basis of the animal data, risk calculations were also performed on the basis of available epidemiological data. A linear relative risk dose-response model was used for analysis of the human data:

$$O = E(1 + \alpha * d),$$

where O is the observed number of liver tumors, E is the expected number of such tumors apart from any exposure, d is a cumulative dose metric (see discussion below) and α is a potency parameter that can be estimated by maximum likelihood techniques. Then it follows that the lifetime probability of liver cancer, P(d), can be estimated by

$$P(d) = P_0(1 + \alpha * d),$$

where P_0 is the background probability of liver cancer death. Actually, the lifetime risk should be estimated by a lifetable method, but the above approximation should be close enough for the purpose of these comparative potency estimates.

Now suppose that for a particular exposure scenario (e.g., a VC atmospheric concentration of 50 ppm, 8 hours a day, 5 days per week), the PBPK model predicts an average daily internal dose metric of X. Then the cumulative exposure that should be used in the dose-response model is X*Y, where Y is the number of years of such exposure. Note that to compute this PBPK-based cumulative dose, we must have an estimate of the "typical" workplace exposure concentration for each subcohort, separate from the number of years of exposure for the subcohort, rather than just a cumulative dose estimate. Only after the internal dose has been calculated with the PBPK model can the duration of exposure be applied to get a cumulative internal dose.

To obtain pharmacokinetic, human-based risk estimates, the PBPK model was run for the exposure scenario appropriate to each of the selected subcohorts from the studies discussed below. The resulting internal dose metrics were multiplied by the appropriate durations to obtain the cumulative internal doses, which were then input into the relative risk model along with the observed and expected liver cancer deaths for each subcohort to get an estimate of the maximum likelihood estimate and 95% confidence interval for α. Then, to determine the risk associated with a continuous lifetime exposure to 1 ppm for comparison with the animal results, the PBPK model was run for a 1 ppm continuous exposure, and the average daily value of the various internal dose metrics was calculated. Multiplying the dose metrics by 70 years gives the appropriate cumulative dose for the relative risk model. For P_0 sufficiently small (which it should be for liver cancer in humans), the extra risk for a lifetime exposure to 1 ppm VC will be approximately:

$$P_0 * \alpha * d_1$$

where d_1 = cumulative internal dose for 1 ppm continuous exposure. Using the 95% upper bound on the estimate for α provides a 95% upper confidence limit on the lifetime risk per ppm for comparison with the animal-based results obtained with the LMS model.

Three epidemiological studies that associated increased liver cancer with exposure to VC, and that provide sufficient information to support separate

exposure concentration and duration estimates (as opposed to just cumulative exposure estimates), were selected for this study: Fox and Collier (1977), Jones et al. (1988), and Simonato et al. (1991).

Fox and Collier (1977)

This study is probably the best with respect to providing information about duration of employment for different exposure-level groupings (see their Table 2). We have previously estimated the average exposure levels to be 12.5, 70, and 300 ppm for the low, medium, and high exposure groups, respectively (Clement, 1987); for comparison Chen and Blancato (1989) estimated averages of 11, 71, and 316 ppm. For the constant exposure groups, these concentrations were input into the human PBPK model, assuming 8 hrs/day and 5 days/week exposure, to get average daily internal dose metrics, which were then multiplied by the duration averages (assumed to be 5, 15, and 27 years) to get cumulative doses. For the intermittent exposure groups, exposure for 2 hrs/day, 5 days/week was assumed.

A weighted average was then performed of the cumulative doses within each exposure group (high, medium, and low), across duration of exposure categories and constant vs intermittent groups. This must be done because observed and expected numbers of liver cancers are reported only by exposure group (see their Table 9). The weighting was performed using the number of workers in the various subcohorts (their Table 2).

The resulting weighted dose estimates for each internal dose metric were then input into the relative risk model along with the observed and expected tumors:

Cumulative Dose	Obs.	Exp.
Average low cumulative dose	1	0.75
Average medium cum. Dose	1	0.77
Average high cum dose	2	0.13

The resulting risk estimates are shown in Table 5. The range of risk estimates reflects uncertainty in the appropriate value for P_0, the background probability of death from liver cancer. The lower risk estimate was calculated using the value of P_0 derived in the Fox and Collier study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989). An important factor to be aware of in interpreting these results is that the classification into exposure groups in this study was based on the maximum exposure level that a worker experienced. This leads to overestimation of cumulative exposure, particularly for the workers in the medium and high groups, and therefore a probable underestimation of risk when using the linear relative risk model.

Table 5
Human-Based Risk Estimates

95% UCL Risk / 1000 / ppm	
Fox & Collier (1977)	0.71 – 4.22
Jones et al. (1988)	0.97 – 3.60
Simonato et al. (1991)	0.40 – 0.79

Jones et al. (1988)

This study was an update of the cohort studied by Fox and Collier. Unfortunately, it does not provide as much information about duration of exposure, so the analysis must be limited to the autoclave workers. For those workers, four duration-of-employment categories are given (see their Table 4); in the present analysis estimated average durations of 1.5, 3, 7.5, and 15 years were used. Their Table 1 shows that the autoclave workers had exposures ranging between 150 and 800 ppm at various points in time. A value of 500 ppm was used in the PBPK model (8 hrs/day, 5 days/week) to get the average daily internal doses. The average daily internal doses were then multiplied by the 4 average durations of exposure to get four groups for analysis:

Cumulative Dose	Obs.	Exp.
Low	0	0.07
Mid 1	1	0.08
Mid 2	2	0.08
High	4	0.15

The resulting risk estimates are shown in Table 5. The lower risk estimate was calculated using the value of P_0 derived in the Jones et al. (1988) study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989).

Simonato et al. (1991)

This study has the largest cohort and the most liver cancer deaths (24). Unfortunately, the exposure information may not be as accurate as in the other two studies discussed above, since it was collected from many different workplaces in several different countries, and since the original reporting of the exposure levels was relatively crude (ranges of <50, 50 -499, and $\geq$ 500 ppm). As in the Fox and Collier study, the classification was based on the "highest level to which the workers were potentially exposed." Thus, as with the previous

studies, the estimates of risk from this cohort are probably underestimates of the true risk.

Another problem with the reporting of the results in this study is that the durations of exposure are not cross-classified according to exposure level as was done in the Fox and Collier report. In fact, there is very little information about duration of exposure that would allow estimation of an average value for the entire cohort, let alone the exposure groups. (Note that we can not use the cumulative exposure groupings, as discussed above, because we need to have exposure level separated from exposure duration.) The information in Simonato et al. (1991) Table 2 (person years of observation by duration of employment) was used to estimate an average duration under the following assumption: if the follow-up time does not depend on the duration of employment, then the differences in the person-years of follow-up is due to the numbers of individuals in each duration category. The weighted average (trying different averages for the $\geq$ 20 year group) gives an estimate of 9 years of employment. This duration was used with model-predicted daily dose metrics for average exposure level estimates of 25, 158, and 600 ppm. The cumulative internal doses were input into the relative risk model with the following observed and expected liver cancer deaths:

Cumulative Dose	Obs.	Exp.
Low cumulative dose	4	2.52
Medium cum. Dose	7	1.86
High cum. Dose	12	2.12

The resulting risk estimates are shown in Table 5. Again, the lower risk estimate was calculated using the value of P_0 derived in the Simonato et al. (1991) study, while the higher risk estimate was calculated using an estimate of the lifetime liver cancer mortality rate in the U.S. population (Chen and Blancato, 1989).

A comparison of the results of the analyses of the three sets of data, shown in Table 5, gives some indication of the consistency of the human results. The lifetime risk of liver cancer per ppm VC exposure estimated from the three studies only ranges over about one order of magnitude: from 0.4×10^{-3} to 4.2×10^{-3}. Very similar risk estimates were also obtained with the alternative dose metrics (RISKM and RISKG). Moreover, these estimates are in remarkable agreement with the estimates based on animal data shown in Table 4. However, any confidence produced by this agreement should be tempered by the likelihood, discussed above, that misclassification of exposure in the human studies may somewhat underestimate the true risk at lower doses. Nevertheless, the agreement of the pharmacokinetic animal-based risk estimates with the pharmacokinetic human-based risk estimates provides strong support for the assumption used in this study: that cross-species scaling of lifetime cancer risk can be performed on a direct basis of lifetime average daily dose (without applying a body surface area adjustment) when the risks are based on biologically appropriate dose metrics calculated with a validated PBPK model.

Pharmacokinetic Sensitivity / Uncertainty Analysis

Table 6 shows the normalized analytical sensitivities for the PBPK model used in this analysis. The normalized analytical sensitivity coefficient represents the fractional change in output associated with a fractional change in the input parameter. For example, if a 1% change in the input parameter results in a 2% change in the output, the sensitivity coefficient would be 2.0. In Table 6, the outputs are the dose metrics used in the analysis of angiosarcoma risk. The parameters in the table are defined in Tables 1 and 2. Sensitivity coefficients of less than 0.01 in absolute value were omitted from the table for clarity. None of the parameters display sensitivities significantly greater than 1.0, indicating that there is no amplification of error from the inputs to the outputs. This is, of course, a desirable trait in a model to be used for risk assessment.

It can be seen that of the 24 parameters in the VC model, 10 have essentially no impact on risk predictions based on any of the dose metrics, and only 8 have a significant impact on predictions based on RISK: the body weight (BW), alveolar ventilation (QPC), cardiac output (QCC), liver blood flow (QLC) and volume (VLC), blood/air partition coefficient (PB), the capacity (VMAX1C) and affinity (KM1) for metabolism by CYP2E1, and in the case of oral gavage, the oral uptake rate (KA). As discussed in the description of the PBPK model, all of these parameters could be reasonably well characterized from experimental data. However, the sensitivity of the risk predictions to the human values of these parameters implies that the risk from exposure to VC could vary considerably from individual to individual, depending on their specific physiology, level of activity, and metabolic capability.

The other dose metrics, RISKM and RISKG, are also sensitive to a number of the parameters in the model for the subsequent metabolism of the reactive metabolites, as well as for the GSH submodel (data not shown). Since these parameters could only be identified from data in rats, their values in the other species are uncertain. Given the sensitivity of RISKM and RISKG to these less certain parameters, and the general similarity of risks based on these two metrics to those based on the RISK metric, the use of the RISK metric would seem to be preferable for quantitative risk assessment.

Table 6
Normalized Parameter Sensitivity in Vinyl Chloride PBPK Model

	Rat Gavage (11.9 mg/kg)	Rat Inhalation (50 ppm,4hr)	Human Inhalation (1 ppm, continuous)	Human Drinking Water (1 ppm)
Dose Metric:	Risk	Risk	Risk	Risk
Parameter				
BW	-0.15	-0.26	-0.22	0.03
QPC	-0.08	0.32	0.11	-0.06
QCC	-0.06	0.65	0.44	-0.35
QFC	---[1]	---	---	---
QLC	-0.06	0.64	0.44	-0.35
VFC	---	---	---	---
VLC	-1.03	-0.84	-0.66	-0.69
PB	0.09	0.73	0.88	0.09
PF	--	--	--	--
PS	--	--	--	--
PR	--	--	--	--
PL	--	--	--	--
VMAX1C	0.69	0.11	0.44	0.43
KM1	-0.07	-0.10	-0.44	-0.43
VMAX2C	0.06	--	--	--
KM2	-0.04	--	--	--
KA	0.29	--	--	--
KC02C	--	--	--	--
KGSMC	--	--	--	--
KFEEC	--	--	--	--
GSO	--	--	--	--
KBC	--	--	--	--
KS	--	--	--	--
KOC	--	--	--	--

[1] – less than 0.05 in absolute value

Monte Carlo Uncertainty / Variability Analysis

The sensitivity analysis described above does not consider the potential interactions between parameters; the parameters are tested individually. Also, sensitivity analysis does not adequately reflect the uncertainty associated with each parameter. The fact that the output is highly sensitive to a particular parameter is not important if the parameter is known exactly. To estimate the combined impact of uncertainty regarding the values of all the parameters, a Monte Carlo analysis can be performed. In a Monte Carlo analysis, the distributions of possible values for each of the input parameters are estimated. The Monte Carlo algorithm then randomly selects a value for each parameter from its distribution and runs the model. The random selection of parameter values and running of the model is repeated a large number of times (typically

hundreds to thousands), until the distribution of the output has been characterized.

To assess the impact of parameter uncertainty on risk predictions, a dose-response model must be selected. In this case the linearized multistage model was used, for the reasons discussed earlier. The actual analysis was performed with our software package, PBPK_SIM, which was developed for the Air Force specifically to perform such a Monte Carlo analysis on PBPK models. The PBPK_SIM program randomly selects a set of parameter values from the distributions for the bioassay animal and runs the PBPK model to obtain dose metric values for each of the bioassay dose groups. It then selects a set of parameter values from the distributions for the human and runs the PBPK model to obtain a dose metric value for a specified human exposure scenario. Finally, it runs the linearized multistage model (or other specified risk model) with the animal and human dose metric values to obtain the human risk estimate. This entire process is repeated a specified number of times until the desired distribution of risks has been obtained.

Tables 1 and 2 list the means (preferred values) and coefficients of variation (CV) used in a Monte Carlo uncertainty analysis of the PBPK model. Truncated normal distributions were used for all parameters except the kinetic parameters, which were assumed to be lognormally distributed. The CVs for the physiological parameters were estimated from data on the variability of published values (USEPA, 1988; Stan Lindstedt, 1992, personal communication), while the CVs for the partition coefficients were based on repeated determinations for two other chemicals, PERC (Gearhart *et al*., 1993) and chloropentafluorobenzene (Clewell and Jarnot, 1994). The CVs for the metabolic and kinetic constants were estimated from a comparison of reported values in the literature and by exercising the model against the various data sets to determine the identifiability of the parameters which were estimated from pharmacokinetic data.

The results of the Monte Carlo analysis are shown in Table 7, which lists the estimated risks associated with lifetime exposure to 1 ppm VC in air or 1 mg/L VC in drinking water (assuming a drinking water ingestion rate of 2L/day). In all cases, the risk estimates represent the 95% UCL for risk, based on the LMS model. However, in order to characterize the impact of uncertainty in the pharmacokinetic parameters on the risk estimates, both the mean and the upper 95^{th} percentile of the distribution of UCL risk estimates are shown. Thus the mean value represents the best estimate of the pharmacokinetically-based upper-bound risk for VC exposure, and the 95^{th} percentile provides a reasonable value for the "highest probable" pharmacokinetic risk estimate, considering both pharmacokinetic uncertainty and uncertainty regarding the low-dose extrapolation. In general, the 95^{th} percentiles of the distributions of risk estimates are within a factor of two of the means, indicating that

pharmacokinetic uncertainty/variability is a relatively small contributor to the overall uncertainty in a risk assessment for VC.

Table 7
Mean and 95th Percentile UCL Risk / 1000
For Angiosarcoma Based on the Pharmacokinetic Dose Metric*

95% UCL Risk / 1000		1 ppm Inhalation		1 mg/L Drinking Water	
Animal Route	**Sex/Species**	**Mean/ UCL**	**95th/ UCL**	**Mean/ UCL**	**95th/ UCL**
Inhalation	male mouse	1.89	3.38	0.67	1.18
	female mouse	3.89	6.95	1.39	2.33
Inhalation	male rat	6.80	14.31	2.45	5.60
	female rat	1.90	3.81	0.67	1.37
Oil gavage	male rat	9.45	17.22	3.36	5.72
	female rat	16.35	29.73	5.83	10.54
Diet	male rat	3.26	5.26	1.14	1.64
	female rat	1.15	1.87	0.41	0.60

* dose metric = lifetime-average total amount metabolized per day, divided by volume of liver

Discussion

Giving priority to the animal studies most closely approximating the human route of exposure, the animal-based estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 ppm VC in air is 2.75×10^{-3}, or 1.1×10^{-6} $(\mu g/m^3)^{-1}$, based on the geometric mean of the results shown in Table 4 for the inhalation studies with male and female rats and mice (Maltoni et al. 1981, 1984, 1988). This value is consistent with the range of risk estimates from the epidemiological studies of 0.4×10^{-3} to 4.2×10^{-3} risk per ppm VC, but is roughly a factor of 80 below the inhalation unit risk of 8.4×10^{-5} $(\mu g/m^3)^{-1}$ that has been used by the EPA since 1985 (Table 8). This difference is primarily attributable to the differences in cross-species scaling between the default approach and the pharmacokinetic approach. Similarly, the animal-based estimate of the carcinogenic risk of angiosarcoma from lifetime exposure to 1 μg/L VC in drinking

water (assuming a drinking water ingestion rate of 2L/day) is $6.8x10^{-7}$ $(\mu g/L)^{-1}$, or 0.024 $(mg/kg/day)^{-1}$, based on the geometric mean of the results for the dietary administration of VC in male and female rats (Feron et al. 1981). This value is also roughly a factor of 80 below the unit risk of 1.9 $(mg/kg/day)^{-1}$, or $5.4x10^{-5}$ $(\mu g/L)^{-1}$ that has been in use by the EPA since 1985 (Table 8). However, in this case the difference is primarily due to the use of the incidence of liver angiosarcoma alone in this analysis, as opposed to the total incidence of all liver tumors (angiosarcoma, hepatocellular carcinoma, hepatocellular adenoma, and neoplastic lesions) plus lung angiosarcoma in the EPA risk assessment.

Table 8
Comparison of Cancer Risk Estimates for Vinyl Chloride

Basis	Inhalation (1 $\mu g/m^3$)	Drinking water (1 $\mu g/L$)
Current EPA HEAST Table (EPA 1999)	84.0×10^{-6}	54.0×10^{-6}
Pharmacokinetic Model	1.1×10^{-6}	0.7×10^{-6}
Human Epidemiology	$0.2 - 1.7 \times 10^{-6}$	

Although VC has often been cited as a chemical for which saturable metabolism should be considered in the risk assessment, saturation appears to become important only at very high exposure levels (greater than 250 ppm by inhalation or 25 mg/kg/day orally) compared to the lowest tumorigenic levels, and thus has little impact on the quantitative risk estimates. The important contribution of pharmacokinetic modeling is to provide a more biologically plausible estimate of the effective dose: total production of reactive metabolites in the target tissue. The ratio of this biologically effective dose to the administered dose is not uniform across routes and species. Therefore any estimate of administered dose is less adequate for performing route-to-route and interspecies extrapolation of risk.

In the pharmacokinetic risk calculations presented in this report, no BSA adjustment factor was applied to obtain the human risks. Although this may appear to represent a departure from previous EPA practice in a risk assessment for VC, this marks the first time a pharmacokinetic dose metric has been used. The dose metric was selected to be consistent with the position stated in the interagency pharmacokinetics group consensus report on cross-species extrapolation of cancer (USEPA 1992) that "...tissues experiencing equal average

concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." However, in the only pharmacokinetic risk assessment adopted by the EPA to this date, the pharmacokinetic dose metric used for methylene chloride was also a "virtual concentration" of an as yet unidentified reactive metabolite, and its use similarly appeared to be consistent with the EPA position. Nevertheless, in their actual application of the methylene chloride pharmacokinetic model, EPA chose to adjust the pharmacokinetic dose metric by a BSA scaling factor to consider potential species differences in pharmacodynamics (USEPA 1987).

The risk assessment performed in this study has focused on cancer risk from a continuous lifetime exposure, or at least an exposure over a large fraction of lifetime. Although there are certainly many uncertainties and unresolved issues regarding cross-species extrapolation of lifetime risks, there are even greater uncertainties regarding the extrapolation of partial-lifetime exposures. In particular, studies which have been performed with VC make it evident that extrapolation of partial lifetime exposure is not straightforward with this chemical. For example, in the comparative studies of partial lifetime exposure of rats to VC discussed earlier (Drew et al. 1983), while exposure from 0 to 6 months resulted in a similar tumor incidence to exposure from 6 to 12 months of life, exposure from 0 to 12 months produced a significantly different incidence than would be expected from the sum of the incidences for the two subintervals.

For angiosarcomas, on the one hand, exposure to VC from 0 to 6 months and from 6 to 12 months resulted in incidences of 5.3% and 3.8%, respectively, while exposure from 0 to 12 months resulted in a much higher incidence of 21.4%. For hepatocellular carcinomas, on the other hand, exposure to VC from 0 to 6 months and from 6 to 12 months resulted in incidences of 4.0% and 11.5%, respectively, while exposure from 0 to 12 months resulted in an incidence of only 7.1%. Thus this comparative bioassay does not provide support for a simple relationship of the observed incidence to the fraction of lifetime of the exposure. As discussed earlier, it seems reasonable to assume that newborns, with their higher rate of cell proliferation, should be at greater risk from genotoxic carcinogens, and some studies with VC support this assumption (Maltoni et al. 1981, Laib et al. 1989, Fedtke et al. 1990), although other well-conducted studies with VC do not (Drew et al. 1983). The issue of sensitive populations has never been seriously dealt with in quantitative carcinogenic risk assessment, but it would seem to be an appropriate consideration during risk management for specific potential exposures.

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