TABLE OF CONTENTS

ABSTRACT

Karin F. Baron. A Cancer Risk Assessment of Low Level Vinyl Acetate Exposure Utilizing a Physiologically Based Pharmacokinetic (PBPK) Model

Vinyl acetate (VA) is widely used in the chemical industry for the manufacturing of polyvinyl acetate emulsions, copolymers with ethylene for adhesives, and paper coatings. The International Agency for Research on Cancer (IARC) has classified vinyl acetate as a possible human carcinogen (IARC, 1995). There is sufficient evidence in the literature to indicate the carcinogenic potential to rats exposed by inhalation to vinyl acetate. There is limited epidemiological data to support a possible excess risk for cancer of the respiratory system with long-term exposure to industrial workers. In order to estimate more closely the risk at current occupational levels, a physiologically based pharmacokinetic (PBPK) model was constructed to predict human target tissue concentrations at plausible industrial exposures. These concentrations were then characterized and compared to tissue levels corresponding to doses known to be tumourigenic in animals to arrive at an estimation of human cancer risk. The model demonstrated that daily low level exposures to vinyl acetate pose a minimal threat of cancer risk to workers.

Acknowledgements

I would like to thank Dr. Louise M. Ball for her personal interest, patience and guidance during my study of Environmental Sciences and Engineering. I am also grateful to Dr. Parker K. Reist and Dr. Elaina M. Kenyon for their positive criticism and support during the preparation of this technical report.

A special note of thanks to Michael Easterling for his assistance with MATLAB™.

I especially appreciate my family for their loving support during the typing and re-typing and re-re-typing of this technical report

I would like to acknowledge Reichhold, Inc. for the opportunity they provided me which made this project and my education in Environmental Sciences and Engineering possible.

List of Figures

Figures **page #**

List of Tables

1. Introduction

1.1 Vinyl Acetate

The International Agency for Research on Cancer (IARC) has classified vinyl acetate (VA) as a possible human carcinogen (IARC, 1991). Vinyl acetate is a clear, colorless liquid, with a sweet fruit like odor. VA is widely used in the chemical industry for the manufacturing of polyvinyl acetate emulsions, copolymers with ethylene for adhesives, and paper coatings. There is no evidence that vinyl acetate exists in nature. Short-term human inhalation exposure is documented as producing mild to severe upper respiratory irritation. Vinyl acetate is not documented as causing adverse human reproductive or developmental effects. There is limited epidemiological data that indicates a possible excess risk for cancer of the respiratory system with long-term exposure to industrial workers. Long-term inhalation studies in rats have shown the development of neoplastic and non-neoplastic lesions, especially in the olfactory region, at dose levels of 200 and 600 ppm (Bogdanffy *et al*, 1994a). There is sufficient evidence in the literature to indicate the carcinogenic potential to rats exposed by inhalation to vinyl acetate.

Vinyl acetate is readily hydrolyzed in the blood. Hydrolysis of vinyl acetate yields acetic acid (AA) and vinyl alcohol, which tautomerizes to acetaldehyde (AAldh). This hydrolysis is rapid by catalysis in rat liver and lung microsomes by esterases, especially carboxyl esterase and acetylcholine esterase (Simon *et al*, 1985). Acetaldehyde has been suggested to be the carcinogenic metabolite of vinyl acetate (Plowchalk *et al*, 1997). Acetaldehyde has been shown to be a clastogen whereas acetic acid is known to be a cytotoxin, hence these metabolites are thought to account for the clastogenic and cytotoxic effects respectively, associated with vinyl acetate. Acetaldehyde is the primary metabolite for many other substances, including ethanol. There is an abundance of human data demonstrating the carcinogenic potential of ethanol in humans (IARC, 1988).

1.2 Hypothesis

Knowing that vinyl acetate is readily metabolized into acetic acid and acetaldehyde, and that acetaldehyde induces nasal tumors in rats at high levels of exposure-750 ppm and greater (Morris *et al*, 1993), we hypothesized that low level vinyl acetate exposure will not yield a sufficient amount of acetaldehyde to cause increased cancer risk to human upper respiratory systems.

We will also examine the site of action for VA accumulation in tissues. The epidemiological data tends to indicate two potential sites of action, the lung and the blood, whereas the animal and alternate modeling data indicate a third site, the nasal cavity.

To test this hypothesis a physiologically based pharmacokinetic (PBPK) model will be modified from existing literature. The model will examine all three potential sites of action utilizing the information in the literature and actual data from exposed workers. The model will also examine different dosing schemes as well as biological variations such as breathing rates. The results from the model will be input to assess vinyl acetate induced cancer risk.

1.3 Overview of Project

The exposure data was collected from 1997 to 2000 at three industrial manufacturing sites, sites A, B, and C. Those sites were involved in different aspects of adhesive and coatings applications utilizing vinyl acetate. Each site employee was monitored for approximately 6-8 hours, performing average daily tasks, using vinyl acetate monomer and vinyl acetate polymers.

Bogdanffy *et al*, 1999, Morris *et al*, 1993 and Simon *et al*, 1985, have documented metabolism and tissue dose responses for VA, and this information will be used to establish a whole body model system. Establishing a working model, such as a physiologically based pharmacokinetic model (PBPK), for vinyl acetate will link the known human and animal exposure data with the available physiological parameters to estimate the actual cancer risk of inhaled vinyl acetate.

The Bogdanffy *et al*, 1999, PBPK model developed using elements from previous chronic inhalation studies in mice and rats and modeling data from Frederick *et al*, 1998, to assess vinyl acetate induced neoplastic and non-neoplastic tumors in the olfactory region of rats and man. The Bogdanffy *et al*, 1999, model estimates exposure to vinyl acetate in human nasal anatomy while Frederick *et al*, 1998, modeled full body exposure to acidic vapors. Elements from the models developed by of Bogdanffy *et al*, 1999, and Frederick *et al*, 1998, as well as Plowchalk *et al*, 1997 and Morris *et al*, 1993, will be modified to develop a whole body model and then utilized with actual exposure data to assess inhalation cancer risk to employees of the vinyl acetate emulsions and adhesives manufacturing facility.

2. Background

2.1 General Toxicity Data

Vinyl acetate is documented as being a respiratory irritant at levels above the American Conference of Governmental Industrial Hygienists (ACGIH) recommended threshold limit value of 10 ppm in an eight hour time weighted average. Respiratory irritation is reported to occur at the 10 to 20 ppm level for humans (Deese and Joyner, 1969). The odor threshold for humans is 5 ppm or less depending upon the individual. Olfactory fatigue is reported to occur in a matter of minutes at 20 ppm.

Vinyl acetate is not considered to be a serious skin irritant, nor is it thought to induce dermatitis. Although there are some documented cases of severe irritation of human skin with blister formation and burning of the cornea (Grant, 1986). Serious eye injuries though, are thought to be avoidable with prompt flushing of the affected area. Exposure of dogs for 6 hours a day for 11 weeks indicated irritation and reddening of the eyes at levels between the range of 91 ppm -186 ppm (ACGIH, 1991.).

Vinyl acetate exhibits moderate acute systemic toxicity. "Rats exposed to 2000 ppm experienced eye and nose irritation and respiratory difficulty and had reduced body weight gain. At autopsy, large numbers of macrophages were seen in the lung" (IARC, 1995). Four-hour exposures to a 4000 ppm atmospheric level produce death in 50% of exposed rats (LC_{50}). The LC_{50} for mice exposed for 4 hours is 1550 ppm and for rabbits exposed for four hours is 2500 ppm. Repeated inhalation exposure of rats to 100 ppm for 6 hrs/day, 5 days/week for 3 weeks produced no sign of adverse effects (ACGIH, 1991).

Oral toxicity studies in both rats and mice seem to indicate a low toxic effect. Dosing experiments using rats that received vinyl acetate in drinking water at concentrations ranging from 200-5000 ppm, recorded a decrease in body weight and water consumption only at the highest dose (Bogdanffy *et al*, 1994b). Seventeen week old mice receiving 1000-2000 ppm of VA in drinking water demonstrated a slight increase in tumors of the oral cavity, esophagus, stomach and lung (Maltoni *et al*, 1997).

The LD_{50} established for VA in rats by gavage is approximately 2.92 g/kg (Budavari, 1989) and in mice is 1.61 g/kg (ACGIH, 1991).

Experimental data in rats demonstrated no observable maternal or developmental toxicity in drinking water studies at levels up to 5000 ppm, but inhalation of 1000 ppm did appear to retard embryonic growth and increase minor skeletal alterations, such as delayed ossification (Hurtt *et al*, 1995). Multigenerational studies in rats failed to demonstrate a consistent effect on reproduction. Vinyl acetate at levels of 5000 ppm in drinking water of rats, did alter some reproductive parameters, such as fertility and mating, but these responses were inconsistent across a two generation experiment (Mebus *et al*, 1995).

2.1.1 Genetic Effects

The evidence collected by IARC, indicates that vinyl acetate can induce DNA cross-links in isolated human lymphocytes and in isolated rat nasal epithelial cells. There are several studies indicating the ability of vinyl acetate to induce sister-chromatid exchange (SCE) at levels ranging from 0.05 – 2.4 mM. " A 48 hr treatment with vinyl acetate (0.05-1mM) induced a drastic increase in sister chromatid exchanges and (in first division cells) structural chromosome aberrations in cultured human lymphocytes" (Norppa *et al*, 1985). Norppa *et al* also observed similar chromosome damage with 0.125-2 mM treatment of acetaldehyde.

Vinyl acetate in whole-blood cultures produced a clear dose dependent increase in total chromosome aberrations. The sister-chromatid exchange frequency in cells treated with vinyl acetate increased linearly with exposure times up to 24 hours. VA is also documented as inducing SCE, chromosomal aberrations, and micronuclei in human whole blood and in isolated lymphocyte cultures *in vitro* (Maki-Paakkanen and Norppa, 1987).

SCE was induced in Chinese hamster ovary cells exposed to vinyl acetate in both the presence and absence of metabolic activation. "Although vinyl acetate appears to act as a direct mutagen, it is most probably not active as such, as it is known to be hydrolyzed *in vivo* and in cultured cells to acetic acid and acetaldehyde by carboxyl esterases" (Sipi *et al*, 1992). Though VA induces SCE, *in vitro* point mutation assays appear to be negative. Similar genotoxic effects were observed with acetaldehyde. "Because such effects were also induced by acetaldehyde, it has been suggested that the SCE observed with vinyl acetate could be elicited by acetaldehyde as metabolite" (Simon *et al*, 1985). The data also suggests that acetaldehyde has slow turn-over in human lymphocytes *in vitro*, and may accumulate in cells and give rise to sister-chromatid exchange and DNA cross-links (He and Lambert, 1985).

Vinyl acetate failed to induce mutations in *Salmonella typhimurim* or SOS repair functions in *Escherichia coli* (Lijinsky and Andrews, 1980). VA did induce DNA protein cross-links in *Escherichia coli* HB101 in the presence of rat liver microsomes but did not

produce DNA adducts in the livers of rats exposed either orally or by inhalation (IARC, 1995).

2.1.2 Carcinogenicity

The evidence is contradictory in the literature as to whether or not vinyl acetate is a human carcinogen. IARC documented two epidemiological studies, a cohort of 4806 exposed male workers in the United States from 1948-1973 and a case-control study of 29,139 exposed male workers in two separate United States manufacturing sites from 1940-1978. In the cohort study by Waxweiler *et al*, 1999981, the death certificates indicated an excess of lung cancer risk in the exposed population when compared with national rates (mortality ratio of 1.5). The case-control study by Ott *et al.*, reported an increase in risk of exposed workers to non-Hodgkin's lymphoma (odds ratio, 1.2), multiple mylenoma (odds ratio, 1.6) and lymphatic leukemia (odds ratio, 1.8). VA can not be considered as the only potential carcinogenic agent in these studies due to two confounding factors: multiple chemicals were involved in both manufacturing sites and also the amount of vinyl acetate exposure each individual received was not determined in either study. Chronic inhalation studies in mice have failed to demonstrate a carcinogenic effect (Bogdanffy *et al*, 1994a). Chronic inhalation exposure to rats have shown, especially at high doses, a clear formation of nasal tumors (Bogdanffy *et al*, 1994a). Bogdanffy *et al* suggests "factors including regional partitioning of vapors into respiratory and olfactory tissues, tissue sensitivity, and differential metabolic capacity." could have attributed to the differences observed between mice and rats with respect to tumor formation.

The evidence indicates that the potential carcinogenic component of vinyl acetate is its' metabolite, acetaldehyde (Plowchalk *et al*, 1997). This creates a unique scenario for exposure because acetaldehyde has been indicated as the potential carcinogenic metabolite of many other compounds including ethanol. The most convincing epidemiological data for ethanol induced carcinogenicity is derived from ingestion of alcoholic beverages as a mode of action (IARC, 1988). Inhalation data for ethanol are not readily available in humans. Animal studies have indicated a lack of evidence for alcohol induced cancer.

Acetaldehyde is a ubiquitous air pollutant resulting from the combustion of wood or tobacco (Morris *et al*, 1993). It is classified as a probable human carcinogen by the

U.S. Environmental Protection Agency (EPA) based on data from rat and hamster inhalation studies. The EPA considered the human data on the acetaldehyde to be inadequate to establish it as a known human carcinogen. Chronic acetaldehyde inhalation studies in rats demonstrated the formation of squamous cell carcinomas at dose levels of 750 ppm and higher. Morris *et al* demonstrated that dose levels below 750 ppm did not result in squamous cell carcinoma formation. It is important to note that nearly all carcinogenicity studies on acetaldehyde have indicated the tumor formation is primarily found in the rat nasal olfactory mucosa. This is significant when considering and estimating potential human cancer risk, because rat nasal physiology is very different from human upper respiratory physiology. These differences are discussed in greater detail in a later section of Chapter 2.

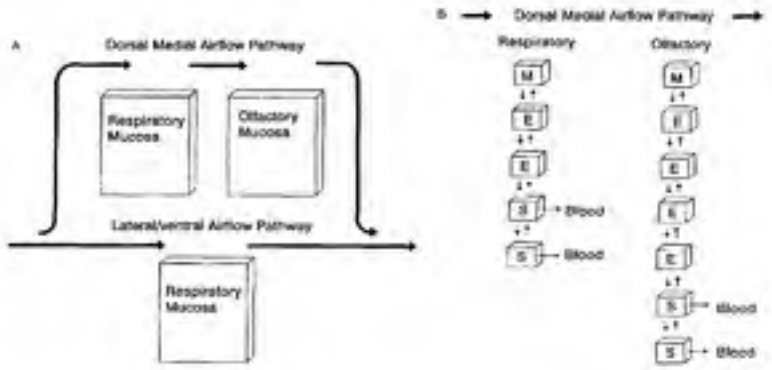
2.2 Current Models

In the current literature there are four PBPK based models that represent the uptake and metabolism of vinyl acetate or similar compounds. Of those four models, only one is scaled to simulate human nasal cavity exposure to VA.

Morris *et al* published the first PBPK model for nasal uptake and metabolism of non-reactive vapors in 1993 (see Figure 1). This model was developed for F344 rats and "incorporates nasal enzyme distribution as well as nasal airflow patterns" (Morris *et al*, 1993). The model assumes two airflow patterns across the nasal cavity; a dorsal medial flow and lateral/ventral airflow. This is due to evidence from studies by Morgan et. al. that demonstrated "the nasal cavity is not uniformly ventilated, (but) rather, inspiratory airstreams follow in a distinct asymmetric pattern which no doubt influences the regional delivered dose of inspired agents" (Morris *et al*, 1993). The nasal compartment is divided into three areas. Each area is further divided into equal depth stacks. Each stack represents different cell types capable of varying degrees of metabolism. The mucous is represented by one stack, two to four stacks represent the epithelial cells and two stacks represent the submucosal space. "It is assumed that the mucous compartment is equilibrated (in accordance with Henry's Law) with the air flowing over it with the blood:air partition coefficient being representative of the mucus:air equilibration" (Morris *et al*, 1993). Each compartment removes vapors by diffusion, metabolism, or circulation and metabolism rates are believed to follow Michaelis-Menten kinetics with respect to compartmental enzyme activity.

Figure 1:

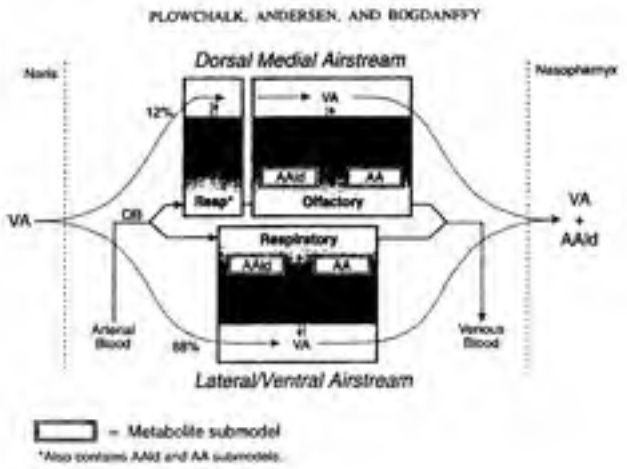
Morris et al Model Describing the Uptake and Metabolism of Non-reactive Vapors in F344 Rats



The second model described in the literature is very similar to Morris *et al* (see Figure 2). Plowchalk *et al*, 1997 described a PBPK model for the uptake, metabolism and intracellular pH changes in the rat nasal cavity when exposed chronically to vinyl acetate. The model was "constructed to describe the deposition of VA in the nasal cavity of the rat and provide estimates of regional tissue exposure to VA, AAld (acetaldehyde) and AA (acetic acid)" (Plowchalk *et al,* 1997). The model structure utilizes descriptions of air flow, blood flow and diffusion similar to Morris et.al.. The model describes the nasal cavity as three separate compartments in contact with two separate air streams. The three compartments are divided into 10 μm thick stacks. The individual stacks are represented by the following cellular structures; the mucosa, epithelial cells, basal cells, and submucosa. The airflow patterns described include the dorsal medial airstream and the lateral/ventral airstream. "The dorsal medial airstream represents the air flowing over only the dorsal medial meatus, which is composed of a small anterior patch of respiratory mucosa and much larger area of olfactory mucosa… The lateral ventral airstream is defined as a composite of airflowing over the dorsal lateral, ventral lateral, ventral media, and middle medial meatuses" (Plowchalk *et al,* 1997). It is assumed that VA will equilibrate with mucous layers as it passes over nasal tissue, and that VA will either be removed by further diffusion, metabolism or taken up into the blood supply. "The extent to which VA can be metabolized within a tissue compartment is dependent upon the complement of enzymes present in the tissue" (Plowchalk *et al,* 1997).

Figure 2:

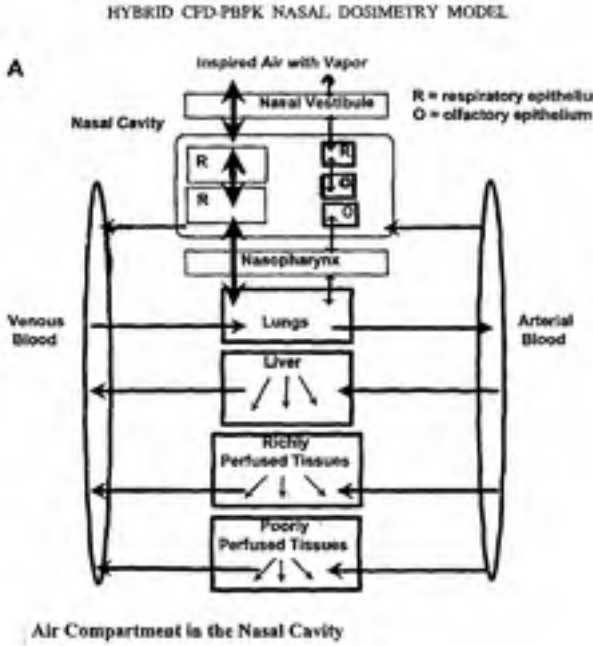
Plowchalk *et al,* Model Describing the Uptake and Metabolism of VA Through the Rat Nasal Cavity



The third model incorporates the whole body system in relation to exposure of acidic vapors (see Figure 3). Frederick *et al*, 1998, designed a "hybrid computational fluid dynamics and PBPK dosimetry model… to estimate the regional tissue dose of organic acids in the rodent and human nasal cavity" (Frederick *et al*, 1998). This model is divided into seven compartments, five of which are in direct contact with both venous and arterial blood flows. The first compartment, the nasal vestibule, is a conduit for airflow only, and neither blood flow nor metabolic activation occurs in this region (Frederick *et al*, 1998). The second compartment is the nasal cavity, which is subdivided by anterior and posterior compartments similar to Morris *et al*. "All air from the nasal cavity then passes over a [third compartment, the nasopharynx] before entering a composite lower respiratory tract compartment" (Frederick *et al*, 1998). The nasopharynx is described similarly to the nasal vestibule, in that neither venous nor arterial blood flow into this region. The lungs are then the next source for contact with the bodies blood flow. The final three compartments are the liver, richly perfused tissues, and poorly perfused tissues, all of which are considered detoxification sites.

Figure 3:

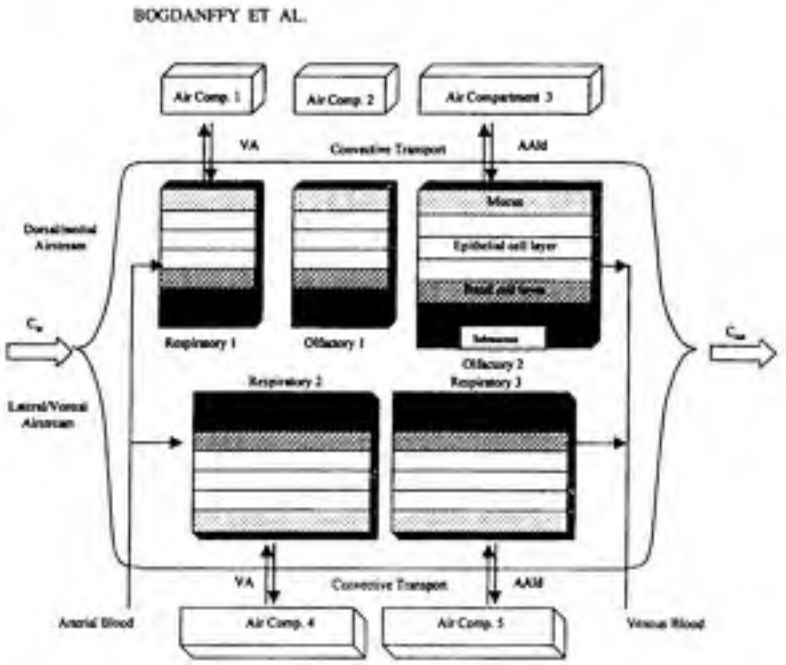
Frederick *et al* Model Describes Uptake and Metabolism of Acidic Vapors in Rats and Humans



Bogdanffy *et al*, 1999, developed the fourth model (see Figure 4). This model is a modified rat nasal model expressing human nasal anatomy and inhalation risk assessment specifically for VA. Though very similar to Morris *et al* and Plowchalk *et al*, this model is compartmentalized into five sections for the rat and four sections for the human with intracellular pH considerations. "The five compartment rat model divides the olfactory region into two compartments, a small dorsal anterior compartment and a larger posterior compartment. The respiratory mucosa on the ventral side is also divided into an anterior and a posterior compartment, resulting in five compartments..." (Bogdanffy *et al*, 1999). Each compartment is further divided into stacks representing different cellular types. The human model is divided similarly to the rat, except the olfactory region is not divided into dorsal anterior and dorsal posterior compartments. Both the rat and human model utilize the two-airstream approach previously described by Morris *et al* and Plowchalk *et al*. This model also "incorporates air phase resistance to mass transfer from the lumen to the air:mucus interface... This is an improvement over the previous model by Plowchalk *et al* (which assumed) equilibrium between the air and the mucus phase" (Bogdanffy *et al*, 1999).

Figure 4:

Bogdanffy *et al* Model Describing the Uptake and Metabolism of VA in the Rat and Human Nasal Cavities

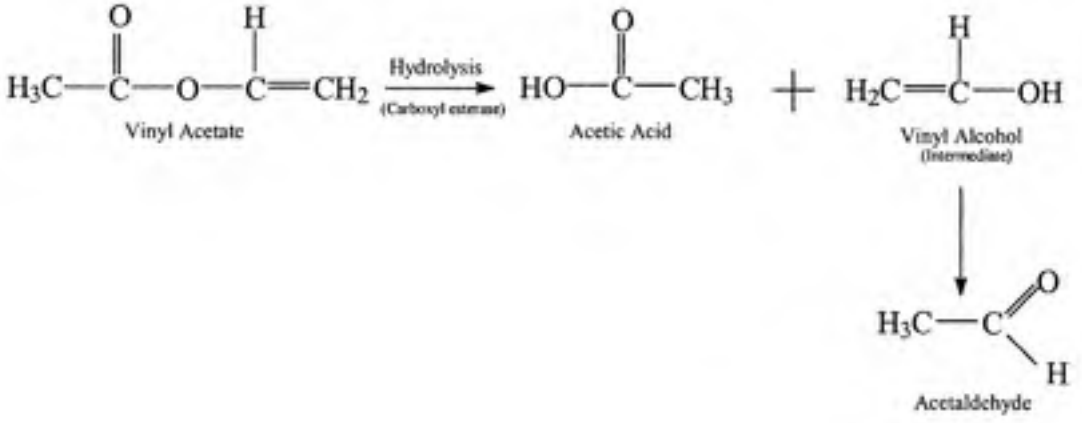


2.3 Metabolism Information

There is very little information available about long term health effects to industrial workers because vinyl acetate is utilized for specific industrial purposes and environmental exposure is unlikely. Similar vinyl halide compounds, like vinyl chloride, have received more attention than vinyl acetate in the literature, due to their demonstrated human toxicity. Vinyl acetate, though, has failed to demonstrate the same level of toxicity as other vinyl halides. Vinyl acetate is however, structurally related to a number of known mutagens and carcinogens, like the vinyl halides. Vinyl halides are thought to be activated to their mutagenic form by means of epoxide formation. It has been suggested that the metabolism of vinyl acetate proceeds by a different mechanism. "By contrast to vinyl halides, the metabolism of which involves epoxide intermediates, vinyl acetate is split by esterases to acetaldehyde and acetic acid" (Simon *et al,* 1985) (see Figure 5).

Figure 5:

Describes the Hydrolysis Reaction of VA that Results in the Formation of AAldh and AA



It has been documented in several *in vitro* reactions that the hydrolysis of VA can occur spontaneously. This reaction appears to always follow first order kinetics. Enzyme mediated hydrolysis is effected by substrate concentrations. "At high substrate concentration (10mM and above) a deviation occurs from the Michaelis-Menten kinetics

observed at lower concentrations" (Simon *et al,* 1985). The data from kinetic experiments with hepatic microsomes, lung microsomes and plasma enzymes demonstrated that all are capable of hydrolysis reactions forming acetic acid from vinyl acetate. The data also indicates a rapid metabolism of VA by esterases mainly in the blood.

The kinetics during inhalation exposure of rats to VA showed both zero-order and first-order patterns depending on the concentration. "This demonstrates that a saturable process must be involved in uptake or metabolism of vinyl acetate" (Simon *et al,* 1985). Metabolic elimination rates were examined and it was found that VA exposure of 650 ppm is metabolized in linear correlation with atmospheric concentration. What Simon *et al* observed were a simultaneous decline in VA during exhalation and a subsequent increase in the acetaldehyde intermediate. Simon *et al* observed decline and subsequent incline is depicted in figure 6.

Figure 6: Exhalation of Acetaldehyde in Two Experiments

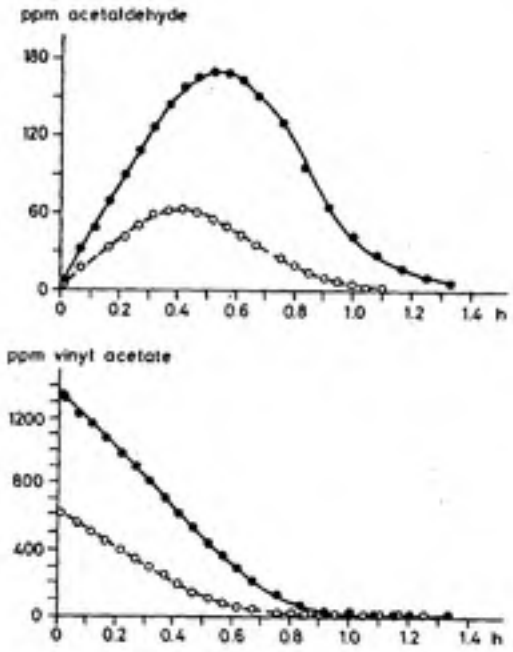


Figure 6: *Bottom panel*: concentration of VA in the gas phase of the closed system. *Top panel*: concentration of acetaldehyde in the gas phase in the same two experiments

2.4 Absorption of Vapors-Anatomy and Physical Properties

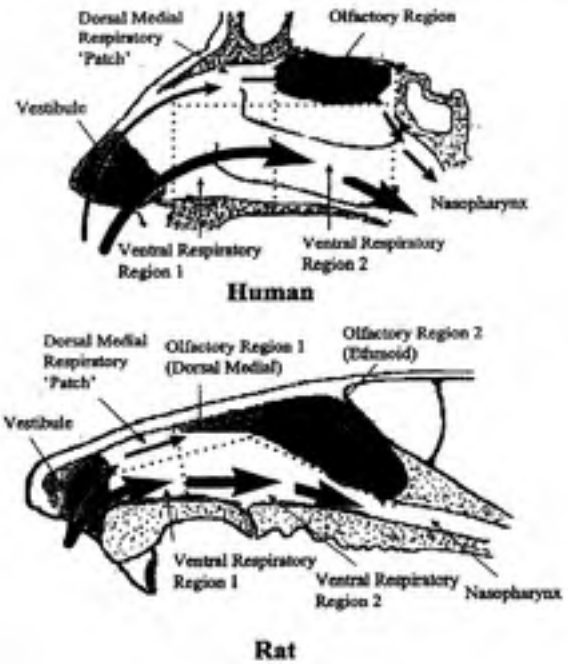
The literature to date utilizes rat exposure data to calculate and predict human risk. There are many differences in basic rat inhalation mechanisms that need to be considered in order to fully assess and predict human risk by these methods. Primarily, absorption of gases and vapors takes place in the lungs, but because these materials must pass through the nasal mucosa, it is possible for these materials to become trapped, especially if the material is water-soluble. The rate of lung absorption is highly dependent upon the rate of respiration and the water solubility of the chemical. "Highly soluble gases such as SO_2 do not penetrate further than the nose and are therefore relatively nontoxic to animals, especially obligatory nose breathers, such as the rat" (Klaassen, 1996). Insoluble gases have the ability to penetrate very deeply in the lung alveoli where they are able to elicit toxic responses. However, very insoluble gases have the ability to pass through the respiratory tract and are taken up by the pulmonary blood supply. Vinyl acetate is fairly soluble, with a water solubility of 20,000 mg/L at 20°C (Riddick *et al* 1985). Acetaldehyde has a much higher solubility in water of $1X10^6$ mg/L at 25°C (Riddick *et al*, 1985).

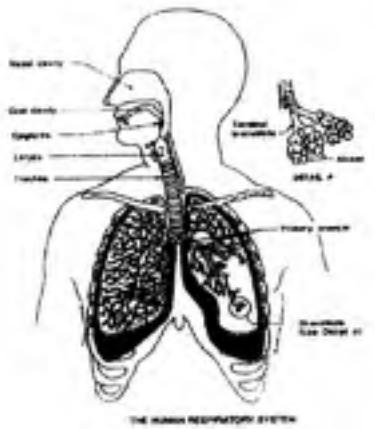
The nose is a crucial site for protection from lung injury. Animals, like rats, that are obligate nasal breathers, are at an increased risk for nasal tumors, compared to humans. The rat nasal compartment is architecturally different from primates, resulting in different airflow patterns. This is an important factor when considering the distribution of lesions produced by chemicals acting locally (Bogdanffy *et al,* 1994a). The rat nasal cavity is also documented as having high metabolic activity. This may also indicate why rats are more prone to develop nasal tumors when exposed to certain chemicals. It is also important to note the variation of mucosal types that comprise the lining of the nasal cavity. "Respiratory and olfactory epithelium each constitute roughly 50% of the total nasal cavity surface area in the adult rat. In contrast, respiratory epithelium covers the majority of the surface of the human nasal cavity and only a small percentage of the total surface area is lined with olfactory epithelium (Bogdanffy *et al*, 1994a).

The nose, mouth, pharynx and larynx anatomically define the upper respiratory tract in humans. The lower respiratory tract is comprised of the trachea, bronchi, alveoli, and the lungs and is the site for gas exchange.

Figure 7:

The Architectural Differences Between the Rat and Human Nasal Cavities (right) and An Overall Picture of the Anatomy of the Human Respiratory System (left)





Absorption of gases into the lungs primarily occurs as molecules diffuse across the alveoli into the blood. "At equilibrium, the ratio of the concentration of chemical in the blood and the chemical in the gas phase is constant. This solubility ratio is called the blood-to-gas partition coefficient. This constant is unique for each gas" (Klaassen, 1996). This only applies to the ratio not the concentration, which is dependent upon Henry's law. The gas concentration in the air versus the blood is proportional, the ratio however, remains unchanged, unless saturation has been reached.

2.5 PBPK

Physiologically based pharmacokinetic models are increasingly utilized for risk assessment. "PBPK models attempt to describe the individual processes that regulate chemical disposition, including tissue partitioning, organ volumes, and blood flow rates as well as biochemical constants for metabolism and protein binding" (Andersen *et al*, 1993). The basic unit of the model is a compartment or combination of compartments where each compartment represents a single region of the body, and is often depicted as a box. These compartments are then connected as a "series of parallel units by means of arterial and venous blood flow" (Andersen *et al,* 1993).

There are several parameters that are then required for the model. The most common parameters are anatomical, physiological, thermodynamic and transport (Klaassen, 1996).

Anatomical parameters include the size of each compartment expressed in terms of volume. These anatomical compartments can be as complex as single tissue layers in the nasal cavity or as board as organ systems, like the liver or lungs.

The physiological parameters are blood flow rates to individual compartments, cardiac output or total blood flow rates, alveolar ventilation rates (if the lung is included as a compartment), and finally if the process involves biotransformation or metabolism, rates, V_{max} and K_m, must be used to describe these processes.

"Thermodynamic parameters relate the total concentration of a xenobiotic (C) in a tissue to the concentration of free xenobiotic in that tissue (C_f) " (Klaassen, 1996). For thermodynamic parameters the partition coefficient is essential if measured values for C and C_f are not available. "…Partition coefficients are used to describe the relative solubility of a circulating chemical in specific tissues" (Andersen *et al,* 1993).

Transport parameters are the methods of passage from one compartment to the next. Transport involves processes like passive diffusion, carrier-mediated transport, facilitated transport or a combination of all three. Materials that are fairly water soluble, such as vinyl acetate (VA) and acetaldehyde (AAldh) are likely to diffuse across membranes into the blood supply fairly easily.

It is important to note PBPK parameters are guidelines and specific assumptions apply when the lung is a compartment of the model. Haggard first described these assumptions in 1924. The assumptions include: (1) ventilation is contiguous, (2) airways such as the nasal passages, trachea, and larynx are inert tubes carrying vapors to the pulmonary region, (3) diffusion of vapors is rapid when compared to blood flow, (4) all material disappearing from inspired air appears in the arterial blood, and (5) vapor in the alveolar air and arterial blood within the lung are in a rapid equilibrium (Klaassen, 1996).

The advantages of using a physiologically based model over a classic pharmacokinetic model are that the model can provide a time course of vinyl acetate distribution to organs or tissues, the model has the ability to estimate effects of changing parameters on tissue concentrations, the same model can be utilized across species, and that complex dosing is easily accommodated.

3. Materials and Methods

3.1 Exposure Information

Vinyl acetate is widely used in the coatings and adhesives industry for various applications. During a period of time between 1997 and 2000, several manufacturing sites from the same industrial chemical company participated in random industrial hygiene monitoring for vinyl acetate. The individuals involved in the monitoring were full time employees performing daily tasks that involved low day to day type exposures to vinyl acetate. The employees were monitored for approximately 6-8 hours.

The task involved varied from site to site, depending on the application each site was currently processing. Data collected from Site A involved employees manufacturing polyvinyl acetate homo-polymer for use in latex emulsions and hot melt adhesives. Employees in this plant are exposed to low level free vinyl acetate monomer during quality control type operations. A quality control or Q.C. Technician, for example, would be responsible for day to day management of the reaction from beginning to end. Downstream exposures could occur for those employees treating the wastewater material or packaging the material in the warehouse for shipment.

Industrial hygiene data collected from Site B involved formulators. Formulators charge solid vinyl acetate polymer into heated mixers for blending with esters in hot melt adhesive applications. Exposure occurs when residual vinyl acetate is released from the polymer especially when the polymer comes in contact with heated equipment. Formulators are responsible for adding the material to the mixer, for maintaining and qualifying the materials to completion, and discharging the molten materials onto chiller plates.

Data collected from Site C involved operators. Operators and formulators perform similar duties, except the raw materials operators work with are water-borne emulsions instead of heated vinyl acetate polymers. These aqueous materials are blended with water and various surfactants for use as latex glues in paper mills and bonded fibers.

Vinyl acetate homo-polymer and co-polymers are utilized for reactions and human exposure is limited to less than approximately 1% of unreacted vinyl acetate monomer. All data collected from sites involving tasks where one time potential high-end exposure or random job duties were being performed was eliminated as a resource point

Breathing zone samples were collected on four different occasions at the three different factory sites (Table 1). All sites involved were manufacturing latex emulsions or polyvinyl acetate based adhesives using vinyl acetate as the primary monomer. In some cases, samples were collected by an ORBO 90 sorbent tube connected to a sampling pump. Air was pulled through the tube with the pump, trapping vapors on the surface media in the collecting tube. In other cases, vinyl acetate vapors were collected by passive diffusion across an activated media badge (carbonaceous porous polymer) as recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA). All samples were analyzed by a third party diagnostic company (NATLSCO) following OSHA 51 methodology.

OSHA 51 method involves analyzing the sample collected using a gas chromatograph equipped with a flame ionization detector. Vinyl acetate is collected from the sample device using a methylene chloride/methanol solution. Working vinyl acetate standard are prepared by spiking specific amounts of stock solution (2 ml VA diluted in a 25ml flask to volume with methanol) into separate septum-capped vials containing 1 ml of 95/5 methylene chloride/methanol. Samples are analyzed using a 6-ft X 2-mm i.d. glass column packed with 0.2% carbowax. The carrier gas used is nitrogen (20 ml/min) and the detector gasses are hydrogen (20 ml/min) and air (250 ml/min). The injection volume is 1 µl. The amount of vinyl acetate found on both the front and back sections, of the device are corrected for the amount found on the blank and is used along with the air volume sampled to report results in ppm (760 mm Hg, 25°C) using the following formula:

$$ppm = \mu g\ VA\ (24.46)\ (1) / L\ of\ air\ sampled\ (86.09)\ (DE)$$

Where 24.46 = molar volume of an ideal gas at 760 mm Hg, 25°C

86.09 = molecular weight of VA

DE = desorption efficiency for VA

Table 1:

Results from Breathing Zone Samples for Industrial Workers in Three Manufacturing Sites Using VA

Date of Exposure	Location	Job Description	TWA (ppm)	Sample Time(min)
1997	Site A	Q.C. Technician	0.31	360
		Q.C. Technician	0.627	396
1998	Site A	Q.C. Technician	0.68	not available
		Warehouse operator	0.53	not available
		Waste Water Treatment	0.46	not available
1998	Site B	Formulator	0.71	456
		Formulator	0.69	429
		Formulator	0.48	467
		Formulator	0.54	405
2000	Site C	Operator	0.65	390
		Operator	0.95	416
		Operator	0.27	417

3.2 Model Design and Development

The model represented in Figure 8 was developed utilizing existing literature data and models from Bogdanffy *et al*, 1999, Plowchalk *et al*, 1997, Frederick *et al*, 1998, and Morris *et al*, 1993. The model represents exposure to vinyl acetate and its' metabolites acetaldehyde and acetic acid. It is divided into six compartments with a major emphasis on the upper respiratory system, because the literature indicates that the majority of metabolic activity occurs in the upper respiratory tract. The compartments described include the nasal vestibule, the nasal cavity, the nasopharynx, the lungs, a compartment of other tissues, and finally the liver.

The nasal vestibule is considered as an air passage compartment only. There is no evidence to consider metabolism and blood flow are occurring in this cavity. Vinyl acetate is allowed to pass through the vestibule into the nasal cavity by airflow (Frederick *et al,* 1998).

The nasal cavity is documented as the primary site for metabolism of vinyl acetate to acetaldehyde and acetic acid (Simon *et al,* 1985). The human nasal cavity has a surface area of 160 cm^2, a weight of 32 g including mucosa (International Commission on Radiological Protection or ICRP, 1974) and a blood flow rate of 15 ml/min (Dubois *et al,* 1998).

Similar to the nasal vestibule, the nasopharynx, is considered as a air conduit only, therefore little to no blood contact takes place and metabolism does not occur (Frederick *et al,* 1998).

The lung is the next site for active metabolism of vinyl acetate from two sources, an inhaled portion from the nasopharynx and a blood flow portion from the nasal cavity. The lung volume is considered by the IRCP to be 0.014 (fraction of body weight). The lung weighs 0.76 (percent of total body weight-ICRP, 1974) with a regional blood flow of 2.5 (% of cardiac output-Brown *et al,* 1997). The minute volume is 7.5 L/min resting and 20 L/min for light activity (ICRP, 1974).

After vinyl acetate enters the lungs, it is further metabolized and either taken into the circulation or exhaled. Simon *et al* demonstrated the capability of carboxyl esterases in human plasma to metabolize VA; therefore the venous blood is included as a

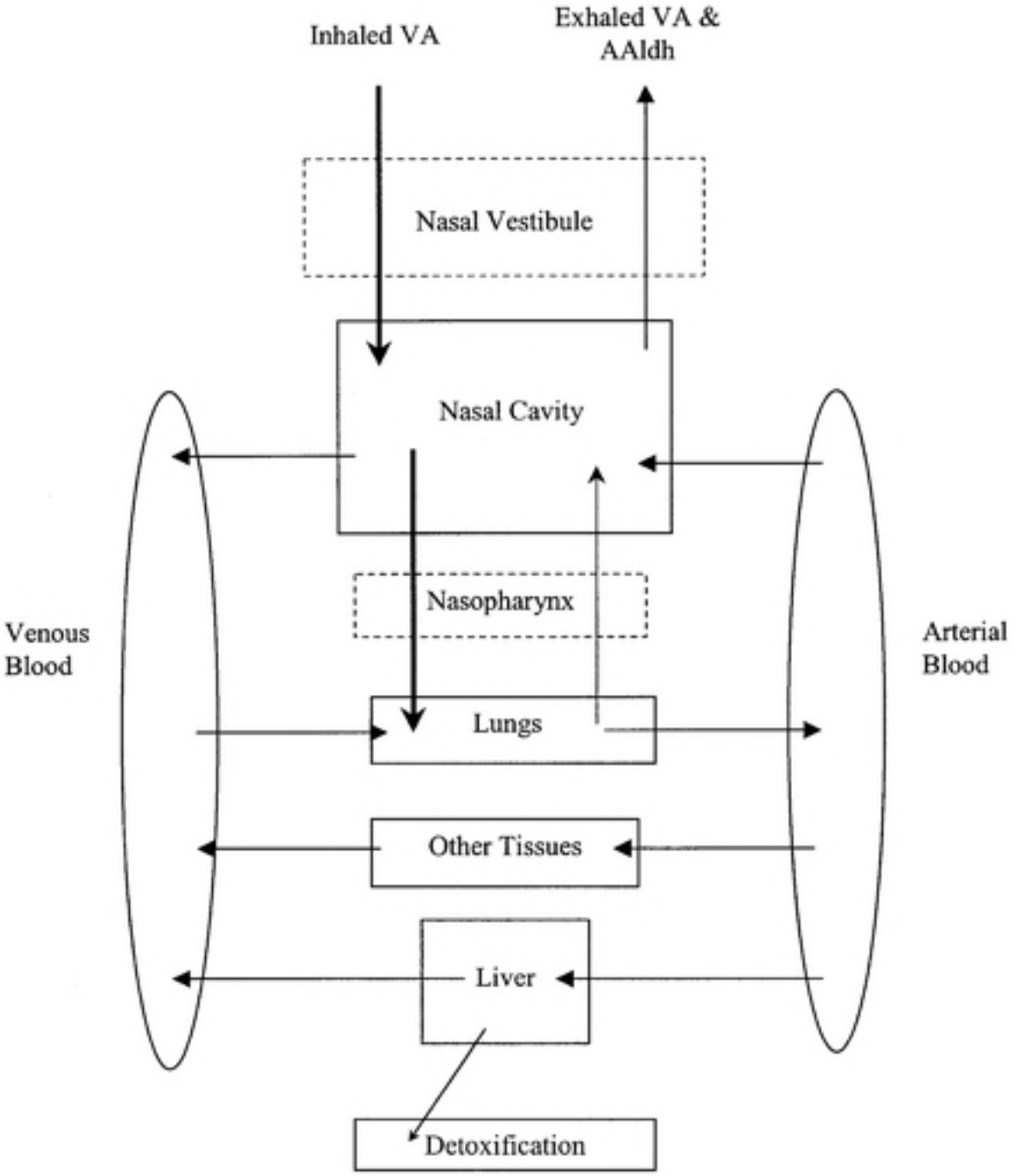
compartment in this model with a volume of 0.046 (fraction of body weight-ICRP, 1974). The arterial blood compartment has a volume of 0.014 (fraction of body weight-ICRP, 1974) and is not considered as a site for metabolism.

Once VA is in circulation, it is believed that very small amounts, if any transition, into the others tissues and the liver compartments. Simon *et al* demonstrated the ability of inhaled VA to be rapidly exhaled as VA and AAldh, suggesting that major deposition in the other tissues is not likely due to the volatility of both compounds. The other tissues compartment has a volume of 0.80 (fraction of body weight-ICRP, 1974) and a cardiac output of 77.3% (calculated from the difference of other compartmental parameters)

It is believed that the liver is the last site for potential metabolism, and is primarily responsible for the final detoxification of acetaldehyde into acetic acid. Hepatic metabolism not included therefore the model demonstrates that any VA that reaches the liver is primarily re-circulated into venous blood. The liver has a volume of 0.026 (fraction of body weight) and a cardiac output blood flow of 22.7% (Williams and Legget, 1989).

Figure 8:

PBPK Model Describing the VA Uptake and Metabolism in Humans



3.3 Model Variables

The model incorporates several aspects of previously documented PBPK models on vinyl acetate and its' metabolites. One aspect of variability exists in the model in that it examines the pathway of only vinyl acetate through each compartment. In order to include acetaldehyde and acetic acid accurately into a PBPK model, it was recommended that several complex sub-models would be necessary. This was not done for the purposes of this paper.

The uncertainty in the model lies within the compartments themselves. VA exposure has not been adequately studied in humans. The data presented for metabolism rates (V_{max} and K_m values) for example, are either based on animal research or measured in human cell types but then scaled according to individual organ size. The blood:tissue partition coefficient was assumed to be 1 throughout the model based on the volatility of vinyl acetate (Brown *et al,* 1997) and a lack of actual human data. The organ volumes and respiratory rates for each compartment is based on a source considered as a reliable references for this type of data (ICRP, 1974). The ICRP values were published in 1974 and the values are rarely re-visited for accuracy.

In considering the lung compartment, it is important to remember, at any time, the lung contains varying amounts of blood and gases. This adds to the potential degree of variability in the model.

The compartment labeled as other tissues is a combination of other organs and tissues such as the skin, kidneys, muscle and fat. This number is simply an addition of available information on various components and does not necessarily reflect all tissues and organs not mentioned in the model.

The consideration of the venous compartment as a site for metabolism is based on data presented by Simon *et al.* It is not typical to include the venous blood as a separate compartment with respect to metabolism. It is typical to consider the unknown chemical to simultaneously mix with blood and blood to be a vehicle only. The decision to then only consider venous blood is based on the overall volatility and amount of vinyl acetate involved in the model. It is estimated that insufficient quantities of vinyl acetate are

available for arterial blood to metabolize. The data from the MATLAB™ results does confirm this belief (see results).

3.4 Model Parameters

The chart below lists the parameters used to describe each model compartment.

PARAMETER	VALUE	SOURCE
Resting Minute Volume (ml/min)	7500	ICRP, 1974
Light activity (ml/min)	20,000	ICRP, 1974
Body weight (kg)	70	ICRP, 1974
Nasal cavity volume [b]	0.00046	Calculated [a]
Lung volume [b]	0.014	ICRP, 1974
Liver volume [b]	0.026	ICRP, 1974
Venous blood volume [b]	0.046	ICRP, 1974
Arterial blood volume [b]	0.014	ICRP, 1974
Other tissues [c]	0.80	ICRP, 1974
Cardiac output (ml/min)		
Resting	5200	Brown (Astrand 1983)
Light Activity	8360	Brown (Astrand 1983)
Nasal cavity(% total output)	0.288	Dubois *et al*, 1998. [d]
Other Tissues(% total output)	77.3	Calculated [e]
Liver (% total output)	22.7	Williams and Leggett, 1989
Metabolic Rates (Michaelis-Menten)		
$V_{max\text{-}NC}$ (mg/min)[f]	7.35	Bogdanffy *et al*, 1999
$K_{m\text{-}NC}$ (mg/ml)[g]	$5X10^{-5}$	Bogdanffy *et al*, 1999
$V_{max\text{-}VB}$ (mg/min)[h]	13,686.2	Simon *et al*, 1985
$K_{m\text{-}VB}$ (mg/ml)[i]	0.611	Simon *et al*, 1985
$V_{max\text{-}LG}$ (mg/min)	223.7	Calculated[j]
$K_{m\text{-}LG}$ (mg/ml)	$1.52X10^{-3}$	Calculated [k]
Partition Coefficients (rat)		
Vinyl acetate (blood:air)	29 +/- 5	Plowchalk *et al*, 1997
Acetaldehyde (blood:air)	224 +/- 43	Plowchalk *et al*, 1997
Acetic acid (blood:air)	80,000	Plowchalk *et al*, 1997
Blood:tissue [l]	1	Brown *et al*, 1997

a – calculated based on ICRP weight of Nasal cavity as 32g (including mucosa), nasal cavity density of 1g/cm^3 and body weight of 70,000g.

b – fraction of body weight (reported in Frederick, reference is ICRP, 1974)

c – addition of muscle, fat and other tissues (0.20 + 0.60) from ICRP, 1974

d – calculated from nasal cavity cardiac output of 15 ml/min and cardiac output of 5200 ml/min

e – calculated by taking the difference from total cardiac output (5200), nasal cavity (15/5200), and calculated cardiac output of liver (22.7%*5200) in ml/min.

f – converted from 441 mg/hr

g – converted from 0.05 mg/L

h - converted from 0.69 µmol/min/mg protein using 72 mg/ml protein content in plasma (Scully, 1986) and venous blood volume of 0.046 (ICRP, 1974)

i – converted from 7.1 mM

j – calculated from Nasal cavity (Bogdanffy *et al,* 1999), scaled to lung organ weight

k – calculated from Nasal cavity (Bogdanffy *et al*, 1999), scaled to lung organ weight

l – if substance is volatile, diffusion occurs rapidly across membranes and it is assumed that the concentration of compounds is in equilibrium, therefore blood:tissue = 1 (Brown *et al,* 1997)

3.5 PBPK Model Rate Equations

The following rate equations were derived to describe distribution and metabolism of vinyl acetate throughout the model compartments.

Rate Equations
Nasal Cavity
$\partial NC/\partial t = Q_P * C_I + C_A * Q_{NC} + Q_P * C_{LG} - (Q_P * C_{NC} + Q_{NC} * C_{NC} + V_{NC} + Q_P * C_X)$
Where: $V_{NC} = V_{max\text{-}NC} * C_{NC} / K_{m\text{-}NC} + C_{NC}$ $C_X = C_{NC}/P_{VA}$
Lung
$\partial LG/\partial t = Q_P * C_{NC} + C_{VB} * Q_C - (V_{LG} + C_{LG} * Q_C + Q_P * C_{LG})$
Where: $V_{LG} = V_{max\text{-}LG} * C_{LG} / K_{m\text{-}LG} + C_{LG}$
Venous Blood
$\partial V/\partial t = C_{NC} * Q_{NC} + C_{OT} * Q_{OT} + C_{LV} * Q_{LV} - (V_{VB} + C_{VB} * Q_C)$
Where: $V_{VB} = V_{max\text{-}VB} * C_{VB} / K_{m\text{-}VB} + C_{VB}$
Arterial Blood
$\partial A/\partial t = C_{LG} * Q_C - (C_A * Q_C)$
Other Tissues
$\partial OT/\partial t = C_A * Q_C - (C_{OT} * Q_{OT})$
Liver
$\partial LV/\partial t = C_A * Q_C - (C_{LV} * Q_{LV})$

3.6 MATLAB™ Model and Programming

NOTE: MATLAB™ programming was completed with the assistance of Michael Easterling

Symbol Definitions:

This chart describes the symbols used in programming the model into MATLAB™

Symbol	Definition	Units
QQ_P	Alveolar ventilation rate	ml/min
C_I	Concentration inhaled	constant (mg/ml)
C_X	Concentration exhaled	mg/ml
C_{NC}	Concentration nasal cavity	mg/ml
C_{LG}	Concentration lung	mg/ml
C_{VB}	Concentration venous blood	mg/ml
C_A	Concentration arterial blood	mg/ml
C_{OT}	Concentration other tissues	mg/ml
C_{LV}	Concentration liver	mg/ml
Q_{NC}	Cardiac output(nasal cavity)	% cardiac output
Q_C	Cardiac output	ml/min
Q_{OT}	Cardiac output (other tissues)	% cardiac output
Q_{LV}	Cardiac output (liver)	% cardiac output
P_{TB}	Partition coefficient tissue:blood	constant
P_{VA}	Partition coefficient blood:vinyl acetate	constant
Vol_{NC}	Volume of nasal cavity	fraction of BW
Vol_{LG}	Volume of lung	fraction of BW
Vol_{VB}	Volume of venous blood	fraction of BW
Vol_A	Volume of arterial blood	fraction of BW
Vol_{OT}	Volume of other tissues	fraction of BW
Vol_{LV}	Volume of liver	fraction of BW

Symbol	Definition	Units
$V_{max\text{-}NC}$	Metabolic rate (nasal cavity)	mg/min
$K_{m\text{-}NC}$	Affinity constant (nasal cavity)	mg/ml
$V_{max\text{-}VB}$	Metabolic rate (venous blood)	mg/min
$K_{m\text{-}VB}$	Affinity constant (venous blood))	mg/ml
$V_{max\text{-}LG}$	Metabolic rate (lung)	mg/min
$K_{m\text{-}LG}$	Affinity constant (lung)	mg/ml

MATLAB™ Programming Text:

The following is the exact text as it was programmed into MATLAB™

```
%MODEL.m file
function varargout = model(t,variables,flag)
switch flag
case ''
  varargout{1} = modeqns(t,variables);
case 'init'
  [varargout{1:3}] = init;
otherwise
  error(['unknown flag ''' flag '''.']);
end
function [tspan, initcond, options] = init

% global statements just share values between functions
global QQP BW QC QNC explength QLV QOT Conc MW
global VolNC VolLG VolVB VolA VolOT VolLV
global VmaxNC KmNC VmaxLG KmLG VmaxVB KmVB
global Pva Pbt

% Initial Conditions
ANC0 = 0;  % initial amount in the nasal cavity
ALG0 = 0;  % initial amount in the lung
AVB0 = 0; % initial amount in venous blood
AA0 = 0; % initial amount in arterial blood
AOT0 = 0; % initial amount in other tissues
ALV0 = 0; % initial amount in liver
```

```
AM0 = 0; %initial amount metabolized

% Biological Parameters
QQP = 7500; % arterial ventilation rate (resting) in ml/min
BW = 70,000,000;  % body weight in mg
QC = 5200; %cardiac output (resting)in ml/min
QNC = 0.288*QC; %cardiac output of nasal cavity in percent of total QC
explength = 480; %length of exposure in minutes
QLV = 0.227*QC; %cardiac output of liver in percent total QC
QOT = 0.773*QC; %cardiac output of other tissues (QC-QLV-QNC) in percent
Conc = 1; %initial concentration in ppm
MW = 86.09; % molecular weight of vinyl acetate
VolNC = 0.00046*BW; %volume of nasal cavity in fraction of body weight
VolLG = 0.014*BW; %volume of lung in fraction of body weight
VolVB = 0.046*BW; %volume of venous blood in fraction of body weight
VolA = 0.014*BW; %volume of arterial blood in fraction of body weight
VolOT = 0.80*BW; %volume of other tissues in fraction of body weight
VolLV = 0.026*BW; %volume of other tissues in fraction of body weight

%Rates
VmaxNC = 7.35; % maximum velocity in mg/min for whole organ
KmNC = 0.00005; %Michaelis-Menten affinity constant in mg/ml
VmaxLG = 223.7; %maximum velocity in mg/min for whole organ
KmLG = 0.00152; %Michaelis-Menten affinity constant in mg/ml
VmaxVB = 13686.2; %maximum velocity in mg/min for whole organ
KmVB = 0.611; %Michaelis-Menten affinity constant in mg/ml

% Partition Coefficients
Pva = 29; % blood/air partition coefficient for vinyl acetate
Pbt = 1; % blood/tissue partition coefficient

tstart = 0.0;  % start time
tstop = 480;   % stop time (minutes)
timestep = 15;  % output every 15 minutes

tspan = [tstart:timestep:tstop];
initcond = [ANC0; ALG0; AVB0; AA0; AOT0; ALV0; AM0;];
options = [];  % empty filler for Matlab

function derivs = modeqns(t,variables)

global QQP BW QC QNC explength QLV QOT Conc MW
global VolNC VolLG VolVB VolA VolOT VolLV
global VmaxNC KmNC VmaxLG KmLG VmaxVB KmVB
global Pva Pbt
```

```
ANC = variables(1);
ALG = variables(2);
AVB = variables(3);
AA = variables(4);
AOT = variables(5);
ALV = variables(6);
AM = variables(7);

% Volume equations for unknown concentrations

CNC = ANC/VolNC/Pbt; %mg/ml
CLG = ALG/VolLG/Pbt; %mg/ml
CVB = AVB/VolVB/Pbt; %mg/ml
CA = AA/VolA/Pbt; %mg/ml
COT = AOT/VolOT/Pbt; %mg/ml
CLV = ALV/VolLV/Pbt; %mg/ml

% Equations

VNC = VmaxNC*CNC/KmNC+CNC; %rate of metabolism in the nasal cavity
VLG = VmaxLG*CLG/KmLG+CLG; %rate of metabolism in the lung
VVB = VmaxVB*CVB/KmVB+CVB; %rate of metabolism in the venous blood
CI = (Conc*MW/24450)/1000; %conversion from ppm to mg/ml
CX = CNC/Pva; % free exhaled concentration calculation using VA partition coefficient
rANC = QQP*CI+CA*QNC+QQP*CLG-(QQP*CNC+QNC*CNC+VNC+QQP*CX);
% rate of change in the nasal cavity(dNC/dt)
rALG = QQP*CNC+CVB*QC-(VLG+CLG*QC+QQP*CLG); %rate of change in the
lung (dLG/dt)
rAVB = CNC*QNC+COT*QOT+CLV*QLV-(VVB+CVB*QC); %rate of change in the
venous blood (dVB/dt)
rAA = CLG*QC-(CA*QC+CA*QC); %rate of change in the arterial blood (dA/dt)
rAOT = CA*QC-(COT*QOT); %rate of change in other tissues (dOT/dt)
rALV = CA*QC-(CLV*QLV); %rate of change in the liver (dLV/dt)
rAM = VNC+VLG+VVB; %total rate of metabolism

derivs(1) = rANC;
derivs(2) = rALG;
derivs(3) = rAVB;
derivs(4) = rAA;
derivs(5) = rAOT;
derivs(6) = rALV;
derivs(7) = rAM;
derivs = derivs'; %matlab book-keeping
```

%RUNMODEL.m file

```
global VolNC VolLG VolVB VolA VolOT VolLV
global Pva Pbt

[t,y] = ode15s('model');
ANC = y(:,1);
ALG = y(:,2);
AVB = y(:,3);
AA = y(:,4);
AOT = y(:,5);
ALV = y(:,6);
AM = y(:,7);

% Volume equations for unknown concentrations

CNC = ANC/VolNC/Pbt; %mg/ml
CLG = ALG/VolLG/Pbt; %mg/ml
CVB = AVB/VolVB/Pbt; %mg/ml
CA = AA/VolA/Pbt; %mg/ml
COT = AOT/VolOT/Pbt; %mg/ml
CLV = ALV/VolLV/Pbt; %mg/ml
CX = CNC/Pva; % free exhaled concentration calculation using VA partition coefficient
```

%MASSCHECK.m file

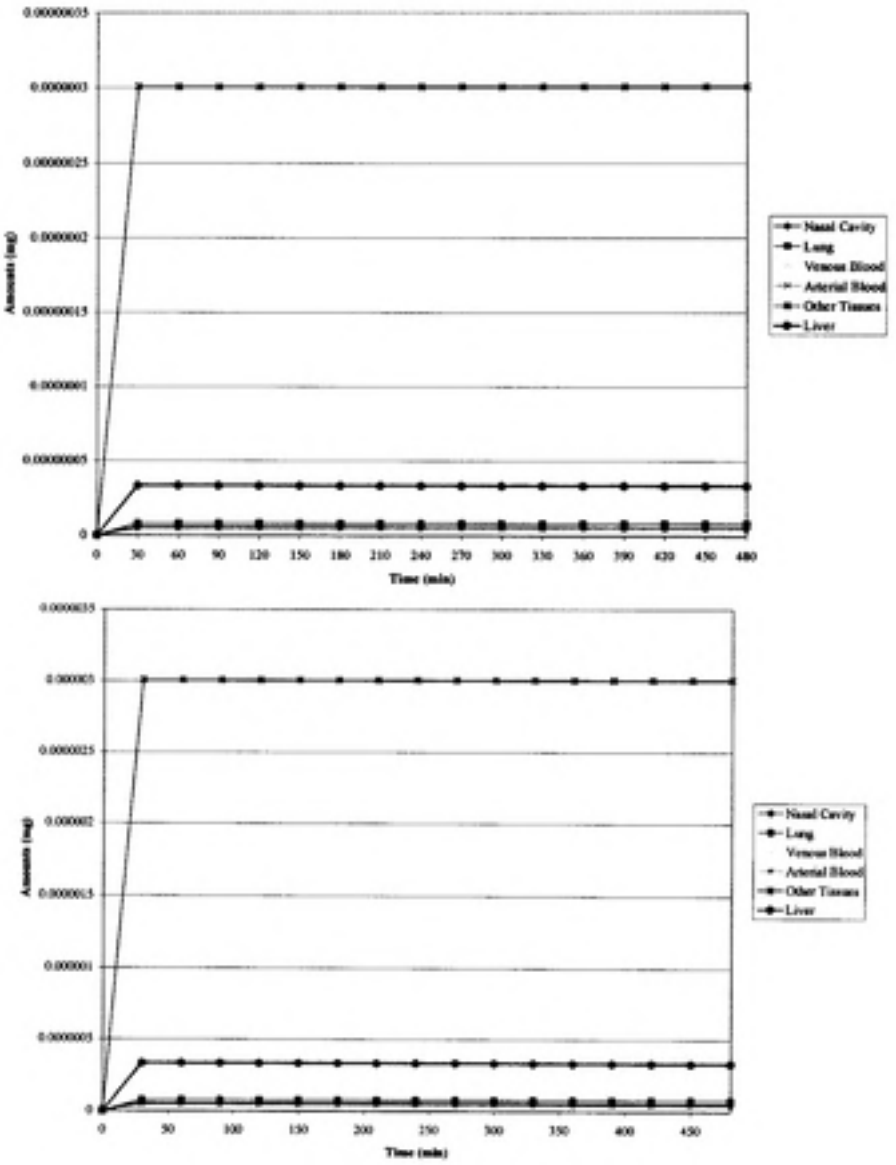
```
% global statements just share values between functions
global QQP BW QC QNC explength QLV QOT Conc MW
global VolNC VolLG VolVB VolA VolOT VolLV
global VmaxNC KmNC VmaxLG KmLG VmaxVB KmVB
global Pva Pbt
Conc = 1; %initial concentration in ppm
MW = 86.09;
CI = (Conc*MW/24450)/1000;
BODY = ANC+ALG+AVB+AA+AOT+ALV+AM
TOTALIN = QQP*CI*480
BALANCE = TOTALIN-BODY-(CX*QQP*480)
```

4. Results

4.1 MATLAB™ Results

The figures below (9-19) represent the output values for each compartment from the MATLAB™ programming presented in the previous section.

Figure 9:Amount in Tissues vs. Time(1 ppm Dose-top, 10 ppm Dose-bottom)



The figures represented on page 36 were derived utilizing the output data from the tables below. Table 2 represents the amount of vinyl acetate in each tissue over eight hours at the 1ppm dosage. Table 3 represents the amount of vinyl acetate in each tissue over eight hours at the 10ppm dosage

Table 2: Amount of VA in Each Tissue Compartment at 1 ppm Dose

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
60	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
90	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
120	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
150	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
180	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
210	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
240	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
270	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
300	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
330	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
360	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
390	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
420	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
450	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08
480	5.456E-09	8.133E-09	3.436E-08	4.066E-09	3.006E-07	3.327E-08

Table 3: Amount of VA in Each Tissue Compartment at 10 ppm Dose

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
60	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
90	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
120	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
150	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
180	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
210	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
240	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
270	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
300	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
330	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
360	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
390	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
420	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
450	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07
480	5.456E-08	8.133E-08	3.436E-07	4.066E-08	3.006E-06	3.327E-07

The following figures (10 and 11) and tables (4 and 5) correspond to the concentrations in tissues over the 8-hour exposure of either 1ppm or 10ppm

Figure 10: Concentration in Tissues vs. Time at 1 ppm Dose

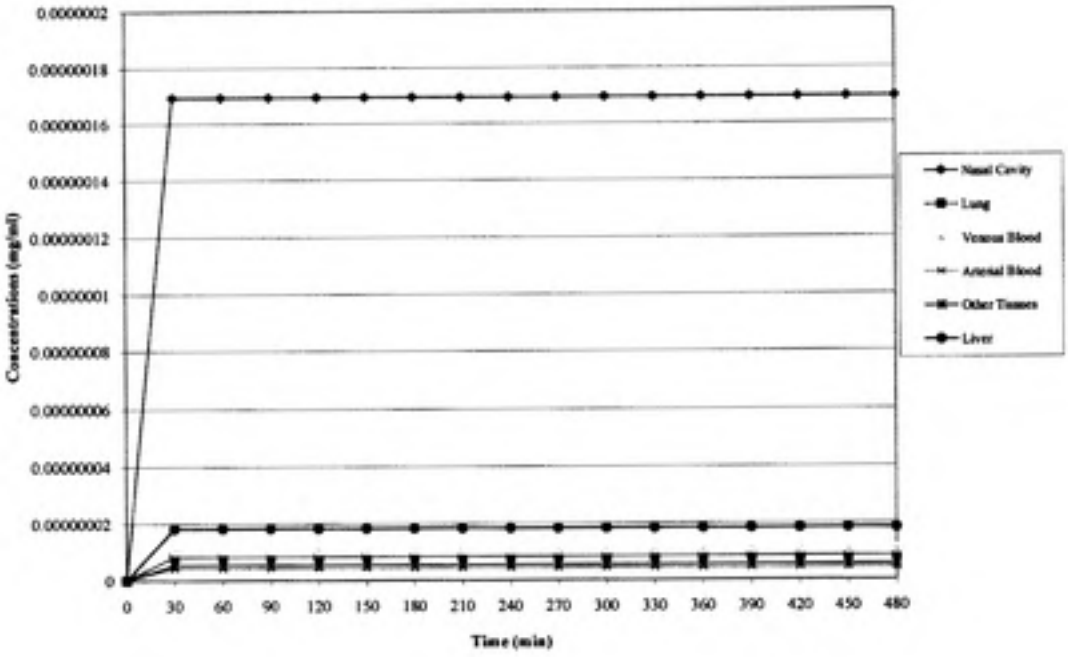
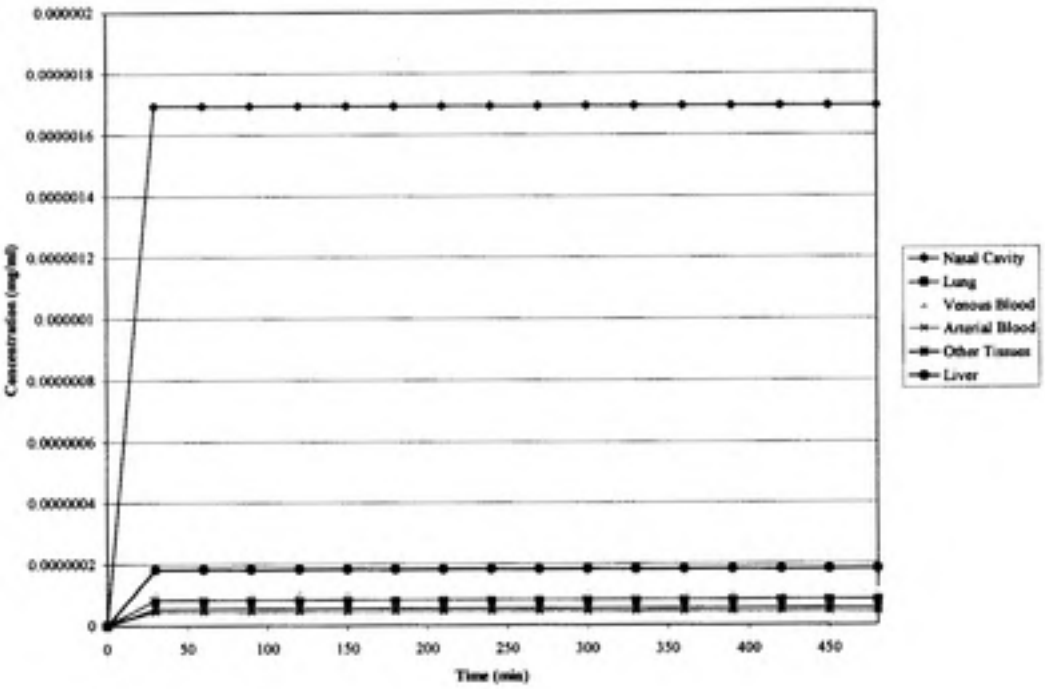


Figure 11: Concentration in Tissues vs. Time at 10 ppm



The figures represented on page 38 were derived utilizing the output data from the tables below. Table 4 represents the concentration of vinyl acetate in each tissue over eight hours at the 1ppm dosage. Table 5 represents the concentration of vinyl acetate in each tissue over eight hours at the 10ppm dosage

Table 4: Concentration of VA in Each Tissue Compartment at 1 ppm Dose

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
60	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
90	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
120	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
150	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
180	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
210	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
240	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
270	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
300	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
330	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
360	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
390	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
420	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
450	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08
480	1.694E-07	8.299E-09	1.076E-08	4.149E-09	5.368E-09	1.828E-08

Table 5: Concentration of VA in Each Tissue Compartment at 10 ppm Dose

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
60	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
90	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
120	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
150	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
180	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
210	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
240	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
270	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
300	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
330	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
360	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
390	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
420	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
450	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07
480	1.694E-06	8.299E-08	1.076E-07	4.149E-08	5.368E-08	1.828E-07

The following figures (12 and 13) correspond to the amounts and concentrations in tissues over the 8-hour exposure of 1 ppm with resting ventilation rates and cardiac blood flow rates replaced to reflect light activity.

Figure 12: Amount in Tissues vs. Time at 1 ppm Dose with Light Activity

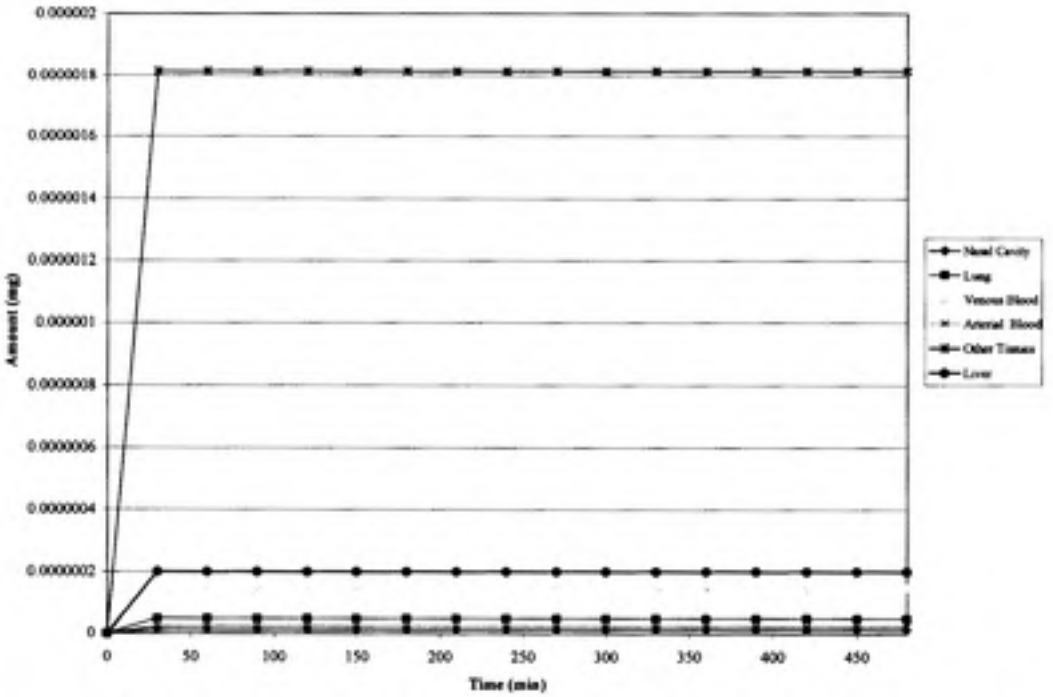
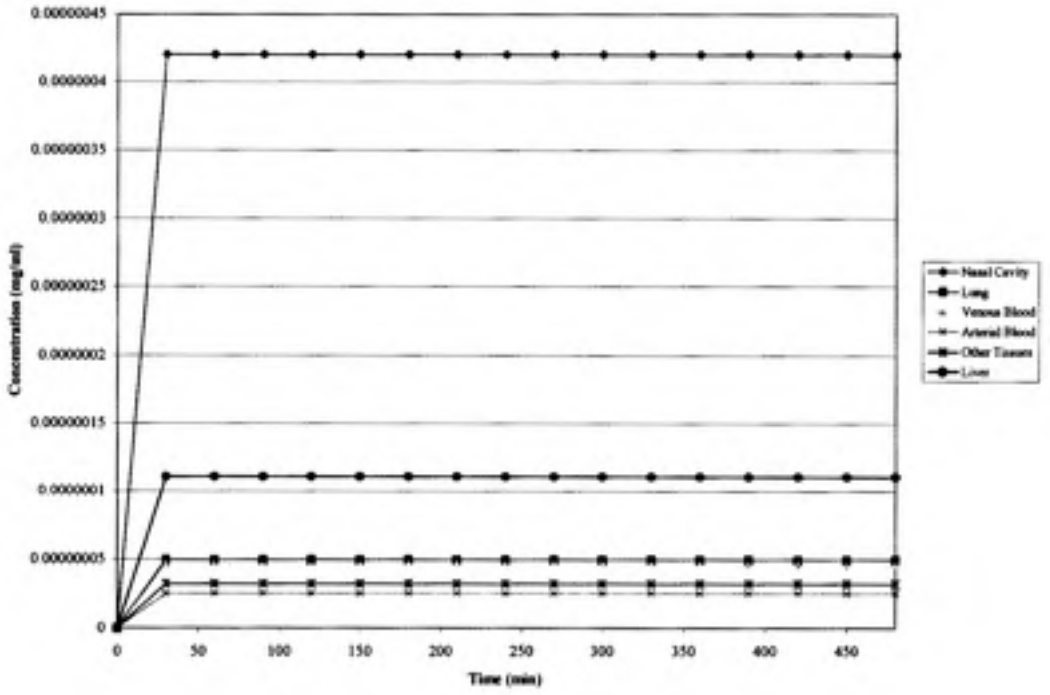


Figure 13: Concentrations in Tissues vs. Time at 1 ppm Dose with Light Activity



The figures represented on page 40 were derived utilizing the output data from the tables below. Table 6 represents the amount of vinyl acetate in each tissue over eight hours at the 1ppm dosage and light activity. Table 7 represents the concentration of vinyl acetate in each tissue over eight hours at the 1ppm dosage and light activity

Table 6: Amount of VA in Each Tissue Compartment at 1 ppm Dose with Light Activity

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
60	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
90	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
120	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
150	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
180	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
210	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
240	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
270	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
300	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
330	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
360	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
390	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
420	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
450	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07
480	1.353E-08	4.91E-08	1.498E-07	2.455E-08	1.815E-06	2.008E-07

Table 7: Concentration of VA in Each Tissue Compartment at 1 ppm Dose with Light Activity

Time	Nasal Cavity	Lung	Venous Blood	Arterial Blood	Other Tissues	Liver
0	0	0	0	0	0	0
30	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
60	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
90	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
120	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
150	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
180	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
210	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
240	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
270	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
300	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
330	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
360	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
390	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
420	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
450	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07
480	4.202E-07	5.01E-08	4.651E-08	2.505E-08	3.24E-08	1.103E-07

The following figures (14-16) represent the concentration exhaled at 1ppm, 10ppm and light activity over the eight hour exposure.

Figure 14: Concentration Exhaled vs. Time at 1 ppm Dose

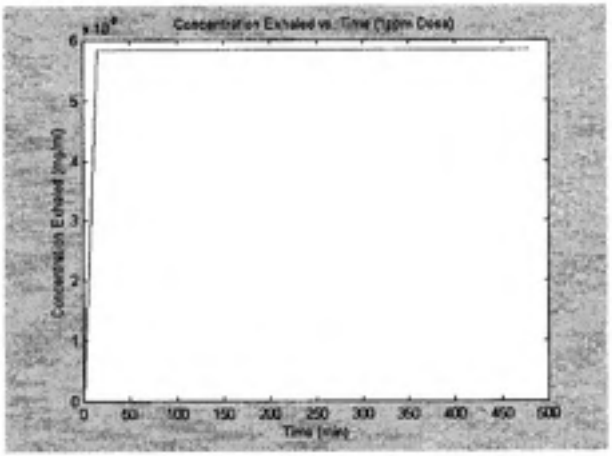


Figure 15: Concentration Exhaled vs. Time at 10 ppm Dose

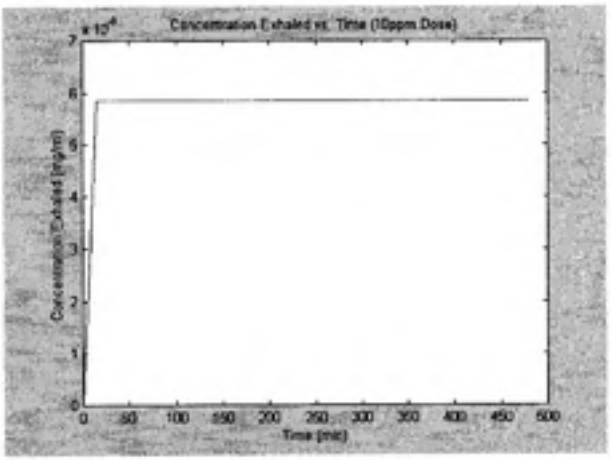
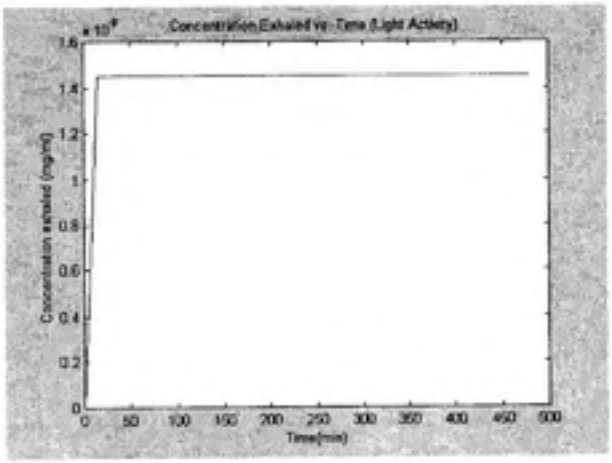


Figure 16: Concentration Exhaled vs. Time at 1 ppm Dose with Light activity



The following figures (17-19) represent the total amount metabolized at 1ppm, 10ppm and light activity over the eight hour exposure.

Figure 17: Total Amount Metabolized vs. Time at 1 ppm Dose

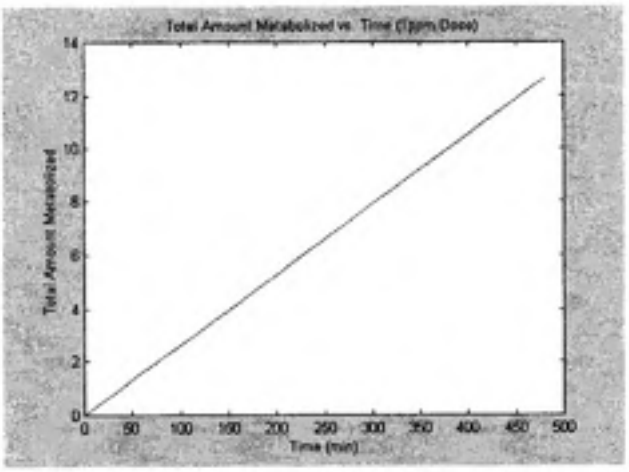


Figure 18: Total Amount Metabolized vs. Time at 10 ppm Dose

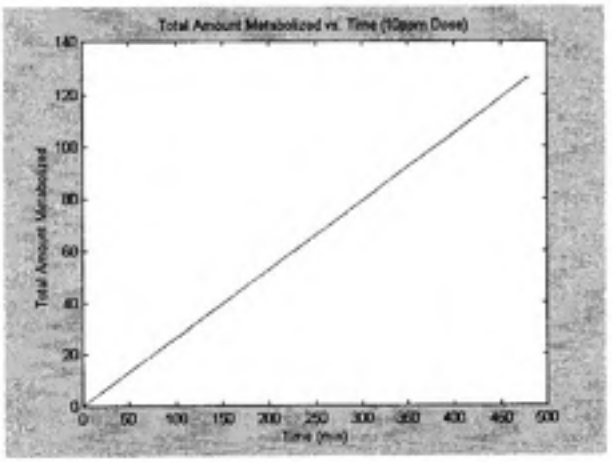
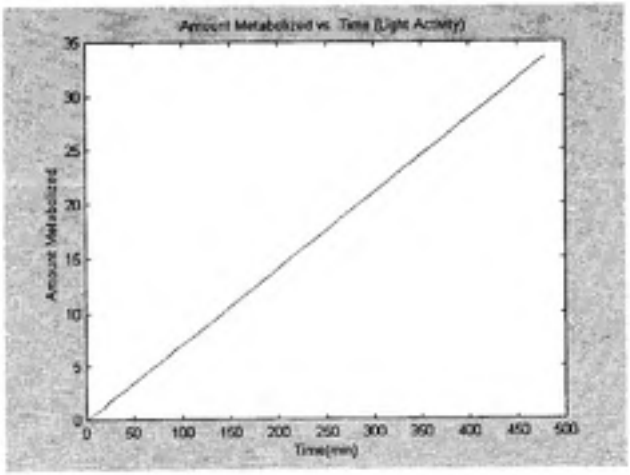


Figure 19: Total Amount Metabolized vs. Time at 1 ppm Dose with Light Activity



4.2 Discussion

The results from MATLAB™ are described in section 4.1. The first set of results is the estimated amount of vinyl acetate in each tissue compartment at two external dosing levels (1ppm and 10 ppm). Figure 9, Table 2 and Table 3 list the amount calculated per compartment. The other tissues compartment appears to contain the largest amount of vinyl acetate at both dose levels followed by the liver, the venous blood the lung, the nasal cavity and the arterial blood, respectively. Altering of the dose level to 10 ppm, results in a factor of 10 increase in the amounts.

When you consider the volume of each compartment to calculate the concentrations of vinyl acetate, the numbers are drastically affected. This is displayed in Figures 10 and 11 and Tables 4 and 5. Considering the volume of the compartment gives a more accurate estimate of tissue deposition. The nasal cavity contains the largest concentration of vinyl acetate followed by the liver, the venous blood, the lung, the other tissues, and the arterial blood, respectively. Again, altering the dose to 10 ppm, results in a factor of 10 increase in concentrations.

The amounts and concentrations in Figures 9-11 and Tables 2-5 were derived using the resting ventilation and cardiac output rates. Figures 12 and 13 and Tables 6 and 7, demonstrate the effect on the amounts and concentrations in each compartment when light activity is considered. This means the ventilation rate was increased from 7,500 ml/min to 20,000 ml/min and the total cardiac output was increased from 5,200 ml/min to 8360 ml/min. This resulted in an overall increase in amounts and tissue concentrations in each compartment of approximately 2.5 times.

The final Figures (14-16) reflect the concentration of exhaled vinyl acetate at the external dose of 1 ppm, 10 ppm and the adjustment to light activity. Figures 17-19 depicts the total amount metabolized at external doses of 1 ppm, 10 ppm and adjusted to light activity. The rate of metabolism always follows a linear increase over time, regardless of the external dosing changes.

4.3 SMUSHT/GLOBAL 86

The nasal cavity concentration from MATLAB™ was programmed into a dose-response curve-fitting program for an estimation of cancer risk. Scientific merging using statistical hypothesis testing or SMUSHT is a program that "implements the likelihood ratio test for the compatibility of 2 data sets to a common multi-stage model" (Howe *et al*, 1986). SMUSHT runs with GLOBAL 86 to calculate and configure risk estimates using various curve-fitting strategies. The MATLAB™ data was compared to previous estimations of internal human dosimetry and rat tumor incidence rates established by Bogdanffy *et al*. He examined not only tumor incidence in rats exposed to external levels of VA (dose ranges were 0, 50, 200 and 600 ppm), but he also noted pathological tissue changes. He then established internal tissue concentrations using a PBPK model and adopted it to address human cancer risk and risk of nasal cavity (specifically olfactory) degeneration. All the SMUSHT/GLOBAL 86 data can be found in the Appendix. The program describes the best curve to fit the data presented then calculates cancer risk levels based on EPA's acceptable lifetime risk of 1 in 1 million. The program selected a two-stage curve to best fit the external dose and the internal concentrations presented and estimated cancer risk. The results are depicted in the table 8 below.

Table 8:

Risk of Cancer Incidence as Predicted by SMUSHT/GLOBAL 86

Dose VA	Risk (1X10-6)	95% CI (p-value)
External (1ppm)	3.6X10-7	0.9596049023*
Internal (CNC= $1.649X10^{-7}$ mg/ml)	8.03X10-7	0.7109948800**

*Chi-squared goodness of fit with 2 degrees of freedom

**Chi-squared goodness of fit with one degree of freedom

The p-values are an estimate of how well the curve selected, fits the data presented. The closer the p-value is to one, the better the curve fit. The table demonstrates a 1 ppm external dose predicts a risk of tumor incidence in rats to be 1 in 36 million (appendix A).

Using the external tumor incidence data and internal nasal cavity concentration estimated from Bogdanffy *et al*, 1999, combined with the internal nasal cavity concentration from our PBPK model, an estimation of human cancer risk was made. Based on the second stage curve (p-value listed in Table 8), the risk of cancer at an internal dose of $1.649X10^{-7}$ mg/ml is predicted to be 1in 80 million (appendix B). This would indicate that a vinyl acetate external human exposure level of 1 ppm per day is considered safe for workers according to both EPA standards and OSHA guidelines.

Our original hypothesis involved cancer risk not only due to vinyl acetate exposure, but also to its' metabolite, acetaldehyde. Considering chemically that 1 mole of vinyl acetate produces 1 mole of acetic acid and 1 mole of acetaldehyde, we can estimate the acetaldehyde risk using the V_{NC} calculated by MATLAB™ and converting it to acetaldehyde concentration in the nasal cavity.

V_{NC} = 0.0249 mg/min

0.0249 / 86.09 = $2.89X10^{-4}$mmol/min

MW (AAldh) = 44 mg/mmol

$2.89X10^{-4}$ mmol/min * 44 mg/mmol = 0.01273 mg/min

Using 6-hour (360 min) exposure and 32 g weight for nasal cavity (converted to ml based on density of 1 g/cm^3 and $1cm^3$ = 1ml)

Concentration of acetaldehyde in the nasal cavity = 0.1432 mg formed in 6 hours/ml of tissue. SMUSHT/GLOBAL 86 predicts the risk of cancer as 1 in 4.62 million (appendix C) with a p-value of 0.7181674171 at 95% CI (Chi-squared goodness of fit with 1 degree of freedom). This value is an estimate based on vinyl acetate chemistry and predicts a risk of cancer well within the OSHA and EPA risk guidelines.

5. Conclusions

5.1 Conclusions

The model developed in this paper was a whole body model of vinyl acetate distribution and metabolism. The outcome demonstrated the likelihood of cancer risk to be well within the range of acceptable risk, but this was only for the concentrations in the nasal cavity. This was due to a lack of data for internal dosing concentrations in other tissue regions such as the lung or the blood. The limitation of only being able to access cancer risk for one target tissue suggests that more research is necessary to access vinyl acetate distribution in other organs, especially the blood. This is important because the epidemiological data presented by IARC indicated a clear increase of risk, in exposed workers, of leukemia and lymphoma related diseases (Ott *et al,* 1989). This is interesting when you consider that vinyl acetate is highly converted over to acetaldehyde by carboxyl esterases, and carboxyl esterases are very predominant in the plasma as indicated by Simon *et al*. This increased risk may be related to the ability of VA and AAldh to invoke DNA cross-links, sister chromatid exchange and structural chromosome damage in cultured human lymphocytes (Norppa *et al,* 1985).

It is difficult to assess vinyl acetate cancer risk in humans. Inhalation studies in rats have indicated a dose-response tumor formation, but all tumors were located in the nasal region. The modeling data to date has concentrated on the respiratory and in particular the nasal olfactory for tumor formation likelihood in humans, but no one has examined the issue of cancer risk in other potential target tissues using a PBPK model. The PBPK model developed in this study does calculate individual compartment concentrations and these concentrations indicate the largest amount of VA is contained in the nasal cavity, but that amount has been shown to be statistically small enough to conclude an extremely low cancer risk to exposed employees. Increasing the dose level to the ACGIH workplace standard of 10 ppm TWA (8 hours) results in an increase in tissue concentrations by a factor of 10. The calculated risk level from our model is now

1in 8.04 million (appendix D) which indicates that the 10 ppm level is well within the OSHA acceptable cancer risk of 1 in 10,000 workers. Bogdanffy *et al*, 1999, also concluded the 10 ppm workplace standard was a conservative level (Bogdanffy *et al,* 1999).

The values estimated for cancer risk were prepared using a ventilation and cardiac output rate for the individual at rest. How does light activity affect the movement of vinyl acetate in the model? The results indicate the concentrations in the nasal cavity are increased 2.5 times. This brings the risk level to 1 in 1.99 million (appendix E), which is still within both the EPA and the OSHA acceptable risk levels for lifetime exposure. It is interesting to note the dramatic change that occurs with the increase from a resting rate to light activity rate. The increase in tissue concentrations is almost entirely attributed to an increased up-take via inhalation from 7500 ml/min to 20,000 ml/min versus the cardiac output increase from 5200 ml/min to 8360 ml/min. Vinyl acetate is thus inhaled at a faster rate, but not circulated in proportion and therefore the model predicts more deposition to tissues. This deposition still falls within acceptable risk factors, but brings about interesting questions regarding the validity of exposure risk models that utilize resting ventilation and cardiac rates as opposed to light activity rates or greater.

The model presented in this paper has demonstrated a reasonable cancer assessment and calculated risk levels that correspond well with existing research on the carcinogenic potential of vinyl acetate. Future research in this area should include improvement on epidemiological data, in particular an in-depth examination of the connection between existing epidemiological data indicating increased risk potential for lymphatic disease and/or leukemia and vinyl acetate workers and clarification of mixed exposure and cancer potential. The two studies available were not able to clarify, nor account for the exact amount of vinyl acetate exposure. This is a common problem in industry when multiple chemicals are being utilized for production purposes.

6. References

ACGIH: Documentation of Threshold Limit Values and Biological Exposure Indices 6th ed., Vol. I, II,III, American Conference of Governmental Industrial Hygienists, Inc., Cincinnati, OH, 1991

Andersen, M.E., Krewski, D., Withey, J.R., "Physiological Pharmacokinetics and Cancer Risk Assessment," Cancer Letters, Apr15;69(1):1-14 (1993).

Bogdanffy, M.S., Sarangapani, R., Plowchalk, D.R., Jarabek, A., Andersen, M., "A Biologically Based Risk Assessment for Vinyl Acetate-Induced Cancer and Noncancer Inhalation Toxicity," Toxicological Sciences, Sep;51(1):19-35 (1999).

Bogdanffy, M.S., Dreef-van der Meulen, H.C., Beems, R.B., Feron, V.J., Cascieri, T.C., Tyler, T.R., Vinegar, M.B., Rickard, R.W., "Chronic Toxicity and Oncogenicity Inhalation Study with Vinyl Acetate in the Rat and Mouse," Fundamental and Applied Toxicology, Aug;23:215-229 (1994a).

Bogdanffy, M.S., Tyler, T.R., Vinegar, M.B., Rickard, R.W., Carpanini, F.M., Cascieri, T.C., "Chronic Toxicity and Oncogenicty Study with Vinyl Acetate: *In-Utero* Exposure in Drinking Water," Fundamental Applied Toxicology, Aug;23(2):206-214 (1994b).

Budavari, S. (ed.), The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., pg. 1575, (1989).

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., "Physiological Parameter Values for Physiologically Based Pharmacokinetic Models," Toxicology and Industrial Health, 13(4): 407-484 (1997).

Deese, D.E., Joyner, R.E., "Vinyl Acetate: A Study of Chronic Human Exposure," American Industrial Hygiene Association Journal, 30:449-457 (1969).

Dubois, A.B., Douglas, J.S., Stitt, J.T., Mohsenin, V., "Production and Absorption of Nitric Oxide Gas in the Nose," Journal of Applied Physiology, Apr;84(4):1217-1224 (1998).

Easterling, M.R., Evans, M.V., Kenyon, E.M., "Comparative Analysis of Software for Physiologically Based Pharmacokinetic Modeling: Simulation, Optimization, and Sensitivity Analysis," Toxicological Methods, 10:203-229 (2000).

Frederick, C.B., Bush, M.L., Lomax, L.G., Black, K.A., Finch, L., Kimbell, J.S., Morgan, K.T., Subramanian, R.P., Morris, J.B., Ultman, J.S., "Application of a Hybrid Computational Fluid Dynamics and Physiologically Based Inhalation Model for Interspecies Dosimetry Extrapolation of Acidic Vapors in the Upper Airways," Toxicology and Applied Pharmacology, Sep;152(1):211-231 (1998).

Grant, W.M., Toxicology of the Eye, 3rd ed., Springfield, Il: Charles C. Thomas Publisher, pg. 978, (1986)

He, S.M., Lambert, B., "Induction and Persistence of SCE-inducing Damage in Human Lymphocytes Exposed to Vinyl Acetate and Acetaldehyde *In-vitro*," Mutation Research, Dec;158(3):201-208 (1985).

Howe, R., Crump, K., Van Landingham, C. "Global-86, A Computer Program to Extrapolate Quantal Animal Data to Low Doses," Prepared for the US EPA, Cincinnati, OH., (1986).

Hurtt, M.E. Vinegar, M.B., Rickard, R.W., Cascerini, T.C., Tyler, T.R., "Developmental Toxicity of Oral and Inhaled Vinyl Acetate in the Rat," Fundamental and Applied Toxicology, Feb;24(2):198-205 (1995).

IARC, "Monographs on the Evaluation of Carcinogenic Risks to Humans," 63:443-465 (1995)

IARC, "Monographs on the Evaluation of Carcinogenic Risks to Humans," 44:153-250 (1988)

International Commission of Radiological Protection (ICRP), No. 23: "Report of the Task Group on Reference Man," Pergamon Press, New York, NY, (1974).

Klaassen, C.D., (ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed., McGraw-Hill, New York, NY, (1996).

Lijinsky, W., Andrews, A.W., "Mutagenicity of Vinyl Compounds in *Salmonella typhimurium*," Teratog. Carcinog. Mutagen., 1(3):259-267 (1980).

Maki-Paakkanen, J. Norppa, H., "Induction of Micronuclei by Vinyl Acetate in Mouse Bone Marrow Cells and Cultured Human Lymphocytes," Mutation Research, Jan;190(1):41-45 (1987).

Maltoni, C., Ciliberti, A., Lefemine, G., Soffritti, M., "Results of a Long-term Experimental Study on the Carcinogenicity of Vinyl Acetate Monomer in Mice," Annuals of the New York Academy of Science," Dec26;837:209-238 (1997).

MATLAB™ Version 5.3 Release 11 Math Works Inc., Natick, MA., (1999).

Mebus, C.A., Carpanini, F.M., Rickard, R.W., Cascieri, T.C., Tyler, T.R., Vinegar, M.B., "A Two-generation Reproduction Study in Rats Receiving Drinking Water Containing Vinyl Acetate," Fundamental and Applied Toxicology, Feb;24(2):206-216 (1995)

Morris, J.K., Hassett, D.H., Blanchard, K.T., "A Physiologically Based Pharmacokinetic Model for Nasal Uptake and Metabolism of Nonreactive Vapors," Toxicology and Applied Pharmacology, 123:120-129 (1993).

Norppa, H., Tursi, F., Pfaffli, P., Maki-Paakkanen J., Jarventaus, H., "Chromosome Damage Induced by Vinyl Acetate Through In-vitro Formation of Acetaldehyde in Human Lymphocytes and Chinese Hamster Ovary Cells," Cancer Research, Oct;45(10):4816-4821 (1985).

Ott, M.G., Teta, M.J., Greenberg, H.L., Lymphatic and Hematopoietic Tissue Cancer in a Chemical Manufacturing Environment," American Journal of Industrial Medicine, 16:631-643 (1989).

Plowchalk, D.R., Andersen, M.E., Bogdanffy, M.S., "Physiologically Based Modeling of Vinyl Acetate Uptake, Metabolism and Intracellular pH Changes in the Rat Nasal Cavity," Toxicology and Applied Pharmacology, Feb;142(2):386-400 (1997).

Riddick, J.A., Bunger, W.B., Sakano, T.K., Techniques of Chemistry, 4th ed., Vol. II. Organic Solvents, New York, NY: John Wiley and Sons, (1985)

Scully, R.E., "Normal Reference Laboratory Values," The New England Journal of Medicine, 314:39-49 (1986)

Simon, P., Filser, J.G., Bolt, H.M., "Metabolism and Pharmacokinetics of Vinyl Acetate," Archives of Toxicology, Aug;57(3):191-195 (1985)

Sipi, P., Jarventaus, H., Norppa, H., "Sister-chromatid Exchanges Induced by Vinyl Esters Respective Carboxylic Acids in Cultured Human Lymphocytes," Mutation Research, May16;279(2):75-82 (1992).

Waxweiller, R.J., Smith, A.H., Falk, H., Tyroler, H.A., "Excess Lung Cancer Risk in a Synthetic Chemicals Plant," Environmental Health Prospectives, 41:159-165 (1981).

Williams, L.R., Legget, R.W., "Reference Values for Resting Blood Flow to Organs of Man," Clin. Phys. Meas. 10:187-217, (1989)

7. Appendices

Appendix A:

SMUSHT/GLOBAL 86 Printout of Animal Data on External Vinyl Acetate Exposure

GLOBAL 86 (JUNE 1990)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT INTERNATIONAL CORPORATION
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

Vinyl acetate external doses

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	50.0000	0/ 60	.50
3	200.000	1/ 60	1.96
4	600.000	7/ 59	5.60

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 1.3757

P-VALUE FOR THE CHI-SQ TEST WITH 2 DEGREES
OF FREEDOM IS .5026485420

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 1.660971895954E-04

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -27.5496167676

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	634.33	373.19	95.0%	Q(0) = .00000 Q(1) = 2.82327E-04
1.00000E-04	.60209	.35422	95.0%	Q(0) = .00000 Q(1) = 2.82327E-04

1.00000E-05	6.02060E-02	3.54201E-02	95.0%	Q(0) = .00000 Q(1) = 2.82327E-04
1.00000E-06	6.02057E-03	3.54200E-03	95.0%	Q(0) = .00000 Q(1) = 2.82327E-04

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	1.66083E-04	2.82287E-04	95.0%	Q(0) = .00000 Q(1) = 2.82327E-04

Vinyl acetate external doses

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	50.0000	0/ 60	.05
3	200.000	1/ 60	.85
4	600.000	7/ 59	7.09

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 8.24673E-02

P-VALUE FOR THE CHI-SQ TEST WITH 2 DEGREES
OF FREEDOM IS .9596049023

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = .000000000000
Q(2) = 3.558234564990E-07

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -26.6417915452

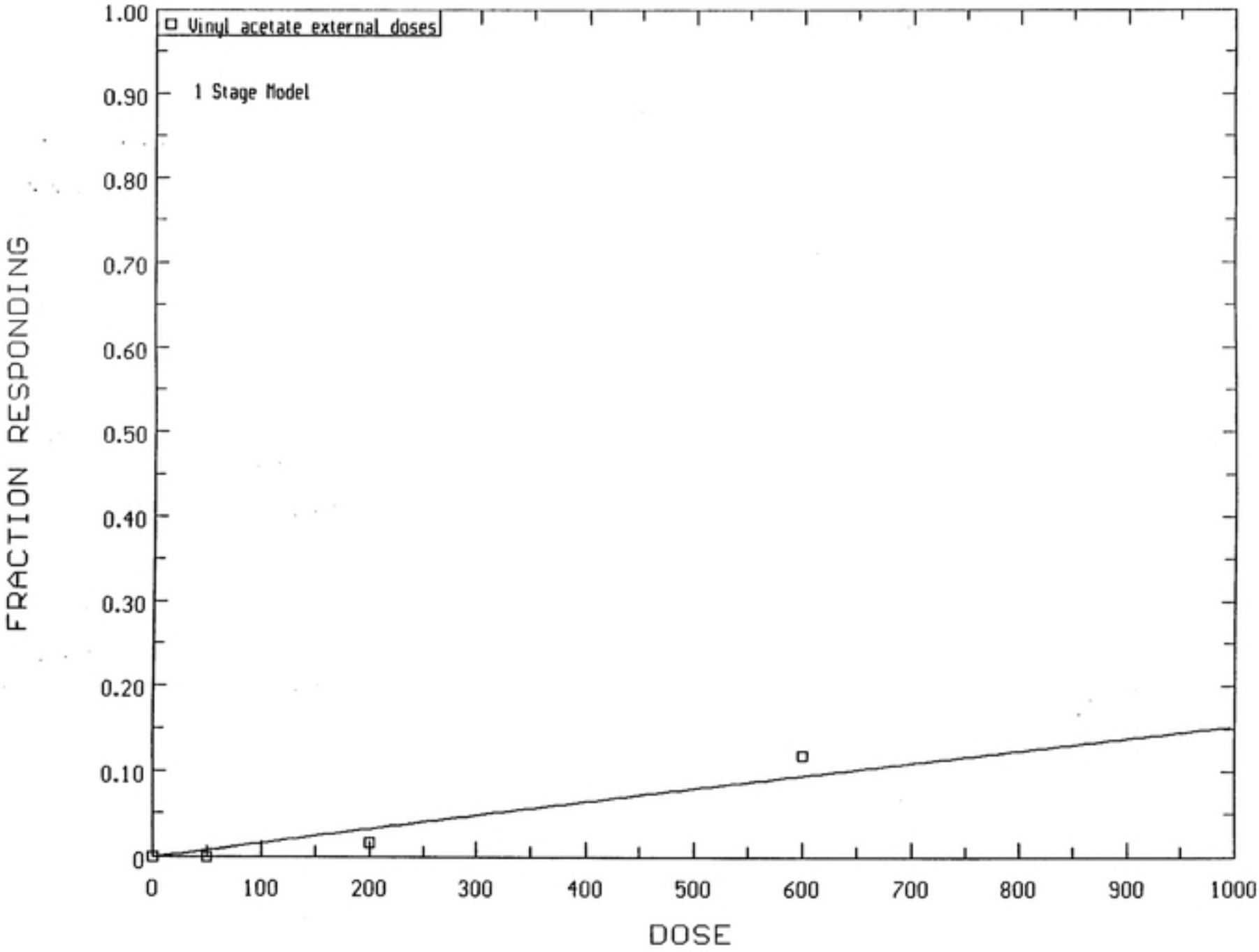
CALCULATIONS ARE BASED UPON EXTRA RISK

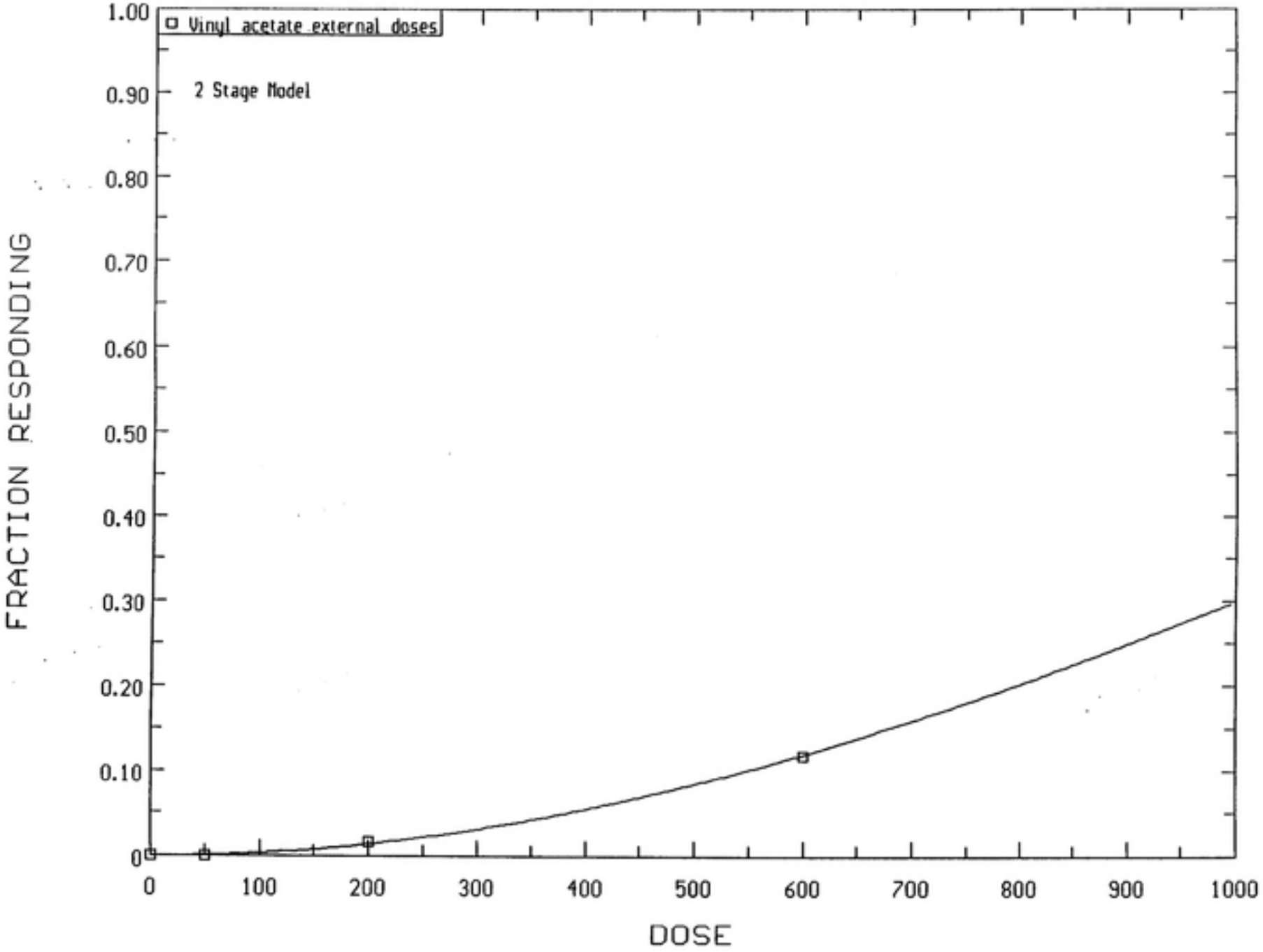
GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	544.15	415.25	95.0%	Q(0) = .00000 Q(1) = 6.84522E-05 Q(2) = 4.46184E-07
1.00000E-04	16.765	.43891	95.0%	Q(0) = .00000 Q(1) = 2.27848E-04 Q(2) = .00000
1.00000E-05	5.3013	4.38892E-02	95.0%	Q(0) = .00000 Q(1) = 2.27848E-04 Q(2) = .00000
1.00000E-06	1.6764	4.38890E-03	95.0%	Q(0) = .00000 Q(1) = 2.27848E-04 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	3.55823E-07	2.27822E-04	95.0%	Q(0) = .00000 Q(1) = 2.27848E-04 Q(2) = -5.49630E-24


Vinyl acetate external doses
1 Stage Model
FRACTION RESPONDING
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
100
200
300
400
500
600
700
800
900
1000
DOSE


Vinyl acetate external doses
2 Stage Model
FRACTION RESPONDING
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
100
200
300
400
500
600
700
800
900
1000
DOSE

Appendix B:

SMUSHT/GLOBAL 86 Printout of Internal Nasal Cavity Concentration from External Dose of 1 ppm Vinyl Acetate

Vinyl acetate internal doses 1 ppm

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	.220000	0/ 60	.08
3	1.11000	1/ 60	.80
4	4.08000	7/ 59	7.12

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .13729

P-VALUE FOR THE CHI-SQ TEST WITH 1 DEGREES
OF FREEDOM IS .7109948800

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 4.736361977654E-03
Q(2) = 6.561231363465E-03

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -26.6820247626

CALCULATIONS ARE BASED UPON EXTRA RISK

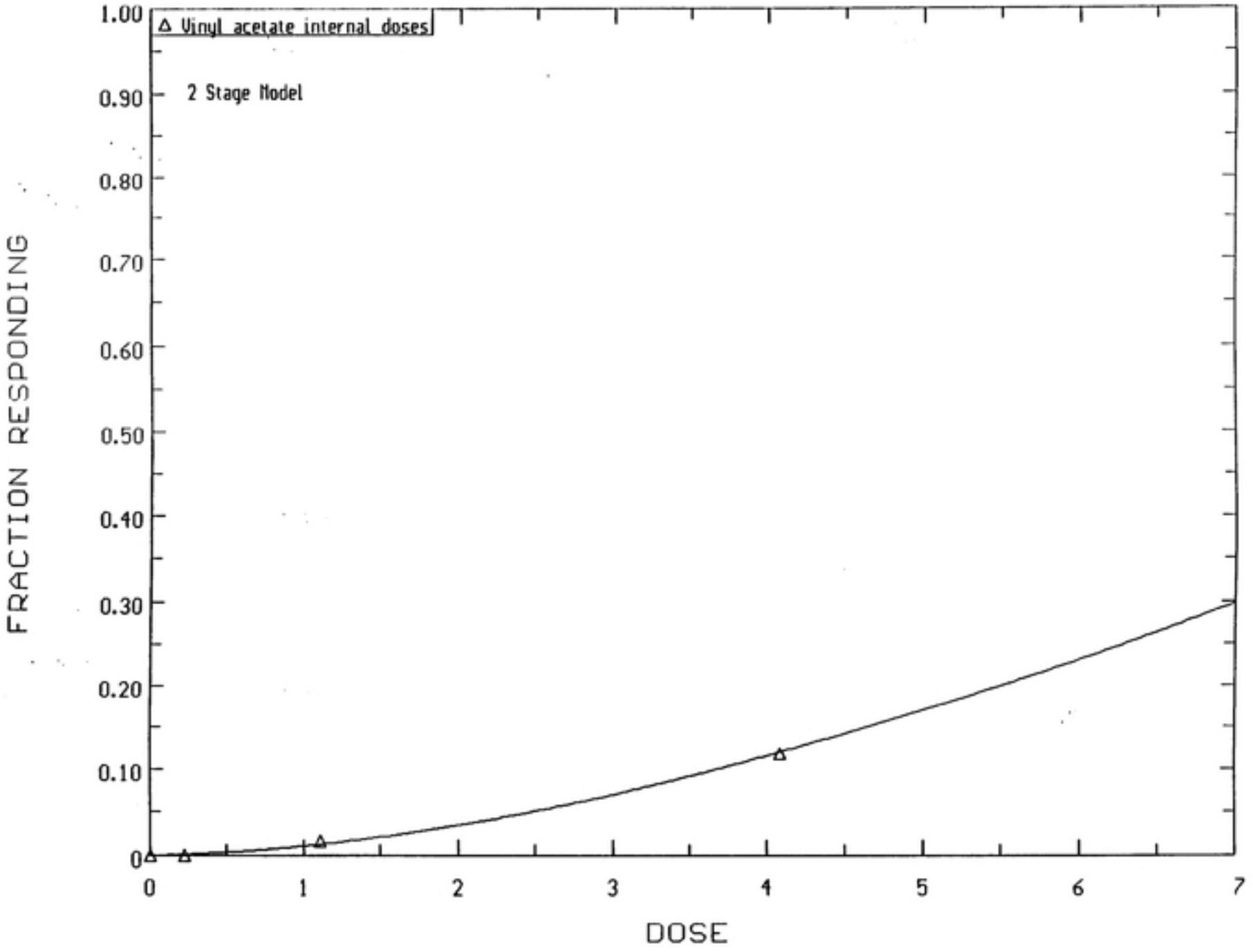
GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	3.6625	2.6178	95.0%	Q(0) = .00000 Q(1) = 3.57317E-02 Q(2) = 1.72498E-03
1.00000E-04	2.05304E-02	2.49347E-03	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-05	2.10520E-03	2.49335E-04	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-06	2.11071E-04	2.49334E-05	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	1.12340E-02	3.93132E-02	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.69400E-04	8.02528E-07	6.79407E-06	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000


Δ Vinyl acetate internal doses
2 Stage Model
FRACTION RESPONDING
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
1
2
3
4
5
6
7
DOSE

Appendix C

SMUSHT/GLOBAL 86 Printout of Internal Nasal Cavity Concentration from External Dose of 1 ppm Acetaldehyde

Acetaldehyde internal dose (external VA 1 ppm)

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	23.0000	0/ 60	.07
3	112.000	1/ 60	.79
4	390.000	7/ 59	7.14

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .13026

P-VALUE FOR THE CHI-SQ TEST WITH 1 DEGREES
OF FREEDOM IS .7181674171

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 3.213327478102E-05
Q(2) = 7.652891380238E-07

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -26.6717559990

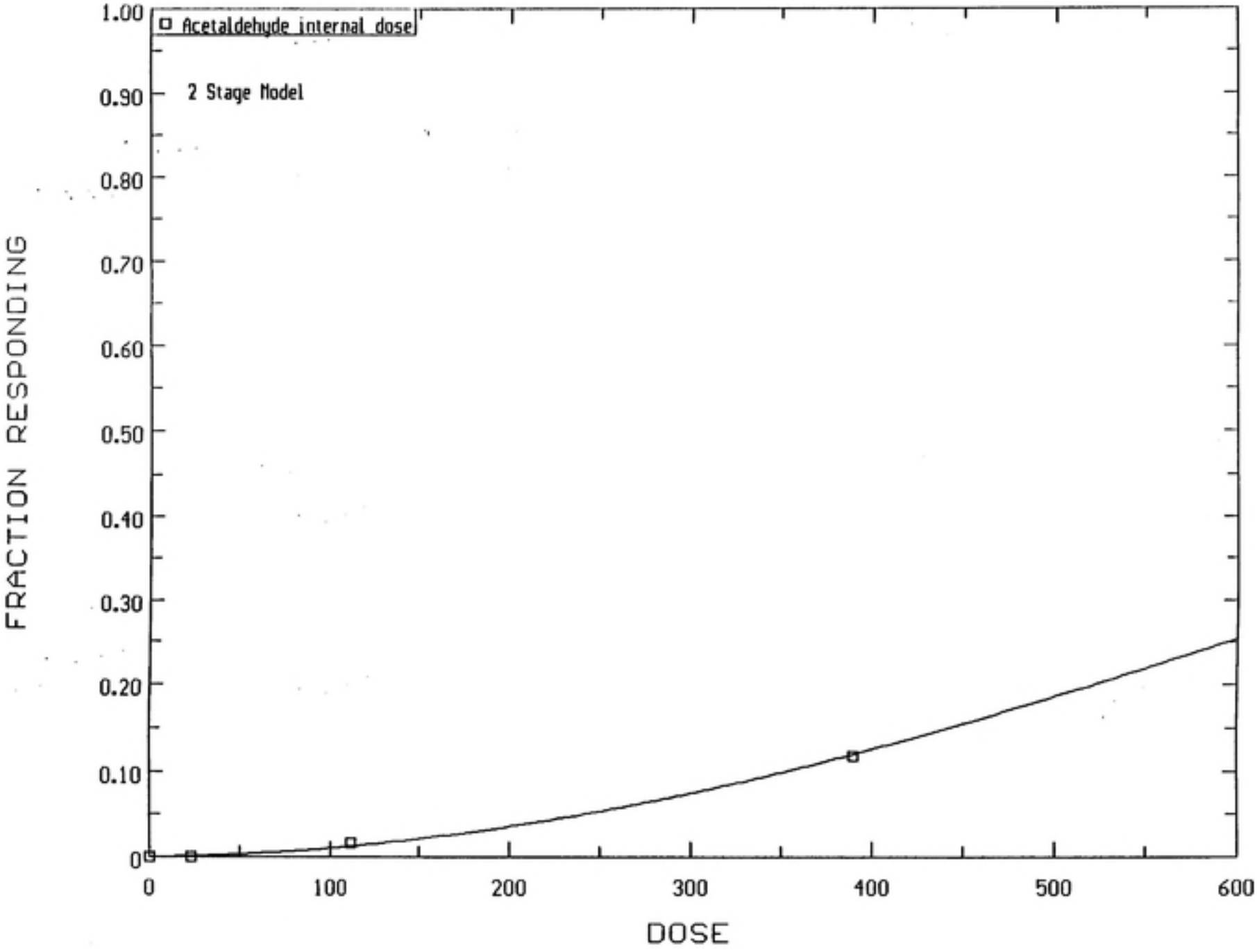
CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	350.64	256.39	95.0%	Q(0) = .00000 Q(1) = 3.07412E-04 Q(2) = 4.03823E-07
1.00000E-04	2.9105	.24715	95.0%	Q(0) = .00000 Q(1) = 4.04634E-04 Q(2) = .00000
1.00000E-05	.30893	2.47138E-02	95.0%	Q(0) = .00000 Q(1) = 4.04634E-04 Q(2) = .00000
1.00000E-06	3.10974E-02	2.47137E-03	95.0%	Q(0) = .00000 Q(1) = 4.04634E-04 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	3.28980E-05	4.04552E-04	95.0%	Q(0) = .00000 Q(1) = 4.04634E-04 Q(2) = .00000
.14320	4.61717E-06	5.79419E-05	95.0%	Q(0) = .00000 Q(1) = 4.04634E-04 Q(2) = .00000


Acetaldehyde internal dose
2 Stage Model
FRACTION RESPONDING
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
100
200
300
400
500
600
DOSE

Appendix D

SMUSHT/GLOBAL 86 Printout of Internal Nasal Cavity Concentration from External Dose of 10 ppm Vinyl Acetate

Vinyl acetate internal doses 10 ppm

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	.220000	0/ 60	.08
3	1.11000	1/ 60	.80
4	4.08000	7/ 59	7.12

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .13729

P-VALUE FOR THE CHI-SQ TEST WITH 1 DEGREES
OF FREEDOM IS .7109948800

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 4.736361977654E-03
Q(2) = 6.561231363465E-03

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -26.6820247626

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	3.6625	2.6178	95.0%	Q(0) = .00000 Q(1) = 3.57317E-02 Q(2) = 1.72498E-03
1.00000E-04	2.05304E-02	2.49347E-03	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-05	2.10520E-03	2.49335E-04	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-06	2.11071E-04	2.49334E-05	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	1.12340E-02	3.93132E-02	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.69400E-03	8.04219E-06	6.79386E-05	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000

Appendix E

SMUSHT/GLOBAL 86 Printout of Internal Nasal Cavity Concentration from External Dose of 1 ppm Vinyl Acetate and Light Activity

Vinyl acetate internal doses light activity

POLYNOMIAL DEGREE SELECTED BY USER
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	0/ 59	.00
2	.220000	0/ 60	.08
3	1.11000	1/ 60	.80
4	4.08000	7/ 59	7.12

CHI-SQUARE GOODNESS OF FIT STATISTIC IS .13729

P-VALUE FOR THE CHI-SQ TEST WITH 1 DEGREES
OF FREEDOM IS .7109948800

FORM OF PROBABILITY FUNCTION :
P(D) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .000000000000
Q(1) = 4.736361977654E-03
Q(2) = 6.561231363465E-03

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -26.6820247626

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.10000	3.6625	2.6178	95.0%	Q(0) = .00000 Q(1) = 3.57317E-02 Q(2) = 1.72498E-03
1.00000E-04	2.05304E-02	2.49347E-03	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-05	2.10520E-03	2.49335E-04	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
1.00000E-06	2.11071E-04	2.49334E-05	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.0000	1.12340E-02	3.93132E-02	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000
4.20200E-04	1.99138E-06	1.68527E-05	95.0%	Q(0) = .00000 Q(1) = 4.01068E-02 Q(2) = .00000