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Dermal Absorption of Methanol and Methanol/Gasoline Mixtures

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

DERMAL ABSORPTION OF METHANOL AND METHANOL/GASOLINE MIXTURES

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Prepared for:

Research Division
California Air Resources Board
2020 L Street
Sacramento, California 95814

Submitted by:

Institute of Toxicology and Environmental Health
School of Veterinary Medicine
University of California
Davis, California 95616

Prepared by:

O.G. Raabe, Ph.D. , Co-Principal Investigator
M.A. Al-Bayati, Ph.D., Co-Principal Investigator
P.D. Shulec, MPH
F. Gielow,
D. Uyeminami,
and
N. Shimasaki

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ABSTRACT

The effect of gasoline concentration on the dermal absorption and fate of ^{14}C-methanol in methanol/gasoline fuel mixtures was studied. A total of 120 adult Sprague-Dawley rats were treated on the intact skin with 100% methanol or one of five methanol/gasoline mixtures containing 95, 90, 85, 50 or 5% methanol. Immediately following dermal application of the methanol/gasoline mixtures, the rats were placed individually in all-glass, flow-through metabolism cages. During the post-treatment period, exhaled and evaporated methanol vapors, exhaled carbon dioxide, and excreta were collected. The animals were sacrificed at intervals following exposure and the ^{14}C-content of excreta, blood and major tissues was determined. The data were used to determine the fraction of applied methanol absorbed for each fuel mixture, to assess the fate and distribution of dermally-applied methanol, and to develop a physiologically-based pharmacokinetic (PBPK) model of methanol disposition in the rat.

The mean dermal absorption efficiency of methanol from the methanol/gasoline fuel mixtures was 24.8, 31.6, 24.5, 27.0, 35.7 and 58.1% for 100, 95, 90, 85, 50 and 5% methanol mixtures, respectively. However, only the fuel mixtures containing 5% and 50% methanol showed statistically significant enhancement of dermal absorption efficiency relative to 100% methanol. One explanation for this enhancement is the increasingly nonpolar nature of the methanol/gasoline mixture as the proportion of gasoline in the mixture increases. At very high gasoline concentrations the increased hydrophobic interactions between the nonpolar gasoline components act to force the very polar methanol out of the vehicle and into the stratum corneum of the skin. Another possible explanation is a skin damaging effect of gasoline which may enhance methanol absorption. Also, simple dilution of the methanol with gasoline would tend to reduce the methanol evaporation rate (and the amount of methanol absorbed) by reducing the methanol partial pressure gradient between the skin surface and the bulk air.

A large percentage (mean of 68%) of the dermally-applied methanol evaporated within 60 minutes for all mixtures studied. With the possible exception of the kidney, there was no elevated concentration of ^{14}C-methanol equivalents in any tissue relative to plasma concentrations during the 24-hour post-treatment period. At 24 hours post-treatment, the main elimination route of absorbed ^{14}C-methanol was via metabolism to carbon dioxide (about 50%). An additional 1-

5% was eliminated via exhalation as methanol and about 4-11% was eliminated as uncharacterized ^{14}C-compounds in the excreta. The remaining portion of absorbed ^{14}C-methanol (30-40%) remained in the body. A PBPK model successfully simulated the main aspects of methanol absorption and disposition as observed in this study.

DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board (CARB). The mention of commercial products, their source, or their use in connection with this report is not to be construed as either an actual or implied endorsement of such products.

ACKNOWLEDGEMENTS

This report was submitted in fulfillment of CARB Contract No. A933-186 entitled, "Dermal Absorption of Methanol and Methanol/Gasoline Mixtures."

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SUMMARY AND CONCLUSIONS

The main objectives of the project and the results were as follows:

1) *Quantitative measurements of the rates of ^{14}C-methanol (as methanol) uptake by the intact skin following the dermal application of methanol alone or when present with gasoline in mixtures at five different concentrations.*

Specific time-based rates of dermal absorption (e.g. mmol/(cm^2-h) or µg/(cm^2-h)) could not be determined because the absorption process was completed well before the first time point at which data was collected. In other words, absorption appeared to be complete in considerably less than 30 min whereas the earliest sample collections were not made until 1 h for all mixtures except the 100% methanol mixture (for which the earliest measurements were made at 30 min). However, the effect of gasoline concentration on the dermal absorption efficiency of methanol (percent of applied methanol dermally absorbed) from methanol/gasoline fuel mixtures was determined. The mean values of the percent of applied methanol dermally absorbed were 24.8, 31.6, 24.5, 27.0, 35.7, and 58.1% for methanol/gasoline fuel mixtures consisting of 100, 95, 90, 85, 50 and 5% methanol, respectively. Only the 5% and 50% methanol mixtures were statistically significantly different (increased) from that for pure methanol. Thus, gasoline concentrations of 50% or greater in methanol/gasoline fuel mixtures enhanced the relative degree to which methanol is absorbed.

It should be noted, however, that while the dermal absorption efficiency of methanol is increased at high gasoline concentrations, the much lower content of methanol in the high percent gasoline mixtures results in a much lower <u>total</u> amount of methanol absorbed. Thus, the total amounts of methanol absorbed per cm^2 of skin surface wetted with each of the mixtures was 120, 140, 110, 110, 86 and 14 µmoles for mixtures consisting of 100, 95, 90, 85, 50 and 5% methanol. From a human health risk assessment point of view, it is the total amount of methanol absorbed systemically following an accidental spill on the skin which is

(2) *Quantitative measurements of tissue uptake and retention of ^{14}C-methanol in brain regions, lung, liver, kidney, blood, and carcass during 24 h following the dermal application of methanol alone or when present with gasoline in methanol/gasoline mixtures.*

Measurements of ^{14}C-methanol equivalents (equiv., includes labeled methanol and metabolites) concentrations were determined only up to 24 h because tissue levels declined to near background at times exceeding 24 h. However, an additional time point was added at 1 h to replace the 48 h time point included in the original study design.

Following application of methanol to the skin, less than 1.0% of the dermally-absorbed dosage was found each in the brain, lung, liver, and kidneys for up to 24 h. Because only a small amount of radioactivity was found in the brain, concentrations of ^{14}C in specific brain regions could not be determined.

Tissue concentrations of ^{14}C-methanol equiv. were in the same range as plasma over the 24 h period. This indicates that most tissues did not concentrate ^{14}C-methanol equiv.. However, in the kidney, no net elimination was observed during the 24 h, suggesting that kidneys may concentrate ^{14}C-methanol equiv. somewhat, probably as formate. Formate is an important toxic metabolite of methanol known to concentrate in the urine.

Although large amounts of ^{14}C-methanol equiv. were present in the body at early time points, only very small total amounts were found in the highly-perfused tissues (liver, brain, lungs, and kidneys). Since it is known that methanol distributes evenly into the total body water, it is probable that the majority of ^{14}C-methanol equiv. in the body was present in the large bulk of muscle tissue (carcass). This is consistent with previously published studies on methanol distribution. Mass-balance calculations showing the vast majority of ^{14}C-methanol equiv. in the carcass (mostly muscle) further support this conclusion. The lack of elevated concentrations of ^{14}C-methanol equiv. in any tissues, particularly the brain, indicates that selective tissue accumulation, per se, is not likely to be a basis for methanol toxicity.

(3) *Measurements of elimination half-life of methanol and metabolites (as ^{14}C-equivalents) in major tissues and in the whole body.*

Half-times for ^{14}C-methanol equiv. from selected tissues and the whole body were estimated assuming first-order elimination kinetics. Mean elimination half-times for plasma, liver, lungs, brain and whole body were 9.7, 16.3, 9.5, 10.4, and 19.9 h. Since no net elimination was observed in the kidney or fat, half-times for these tissues could not be determined in this study.

(4) *Measurements of the fraction of dermally-absorbed methanol excreted in the urine, feces, and exhaled as parent compound and/or metabolites (as ^{14}C-equivalents) as observed during 24 h following treatment.*

Twenty-four hours following dermal application of 100% methanol, the mean fractions of dermally-absorbed methanol excreted in the urine, excreted in the feces, exhaled as methanol, or exhaled as the metabolite carbon dioxide were 7.0, 0.7, 4.9, and 43.4% respectively. The remaining label (30-40%) either remained in the viscera or was lost during sample processing of the carcass (via evaporation as methanol). Relative fractions of dermally-absorbed methanol excreted via these pathways were similar for all applied methanol/gasoline fuel mixtures.

(5) *Measurements of the fraction of applied dosage that evaporates from the treatment sites as observed during 24 h post-treatment.*

For all methanol/gasoline mixtures (including 100% methanol) except those containing 5% and 50% methanol, 72-78% of the applied ^{14}C-methanol evaporated directly from the skin. However, for the 5% and 50% methanol mixtures, a significantly lower amount of the applied methanol was evaporated (48 and 63% respectively). The reduced methanol evaporation rate of the 5% and 50% methanol mixtures may be a function of, or the cause of, the increased dermal absorption efficiency of methanol observed for these mixtures.

Evaporation therefore constitutes an important fate process for methanol upon dermal application and limits the amount of methanol which can be absorbed. Evaporation occurs very rapidly, being complete within at most 30 min for the volume of mixtures used in this study. Since the present study evaluated exposures in a metabolism cage with fixed air flow and moderate temperature, evaporation rates may be higher in an outdoor setting if there is greater air movement or a higher ambient temperature. This may result in reduced dermal absorption of methanol spilled on the skin.

6) *Develop a physiologically-based pharmacokinetic (PBPK) model of methanol disposition in the rat following dermal application of methanol.*

A PBPK model was developed to simulate the dermal absorption and disposition of methanol in the rat. The model successfully simulated the main aspects of methanol disposition in the rat following topical application. However, validation of the model could be improved in future studies by increasing the number of time points (particularly at early times) so that important kinetic processes (particularly methanol absorption, evaporation and elimination) could be quantitatively characterized.

RECOMMENDATIONS

Because methanol appears to be absorbed very rapidly, use of the dermal absorption efficiencies (DAE) estimated here should allow a reasonable approximation of the systemic dose of methanol. However, two important caveats must be noted. First, because evaporation has a dramatic influence on the dermal absorption of methanol, the estimates of methanol DAE measured here may under- or over-estimate actual dermal absorption occurring in the ambient environment depending on air movement. In the most likely case, actual dermal absorption would be less due to generally greater air movement, and hence evaporation, in the ambient environment. Second, the limited available in vitro data for human and rat skin suggest that the dermal absorption of methanol in rats may be up to 8 times greater than in the human. The in vivo rat data collected here are consistent with this conclusion, however, carefully-controlled in vivo human studies are needed in order to verify this supposition.

Estimates of the DAE are critical to estimating the amount of methanol likely to enter the blood following an accidental fuel spill on the skin. However, quantitative health risk assessment of methanol exposures also requires that the systemic dose of methanol be evaluated in terms of its ability to produce toxicity. Since the toxicity of methanol is due primarily to its metabolite formate, it is essential that the metabolic kinetic parameters describing the relative rates of formate production and elimination be determined. The availability of these parameters would allow quantitative prediction of the blood formate level given various accidental doses of methanol, regardless of route of exposure or exposure level. The present lack of quantitative estimates of these parameters for either the rat or the human constitutes the critical data gap preventing completion of the physiologically-based pharmacokinetic model developed here and its extension to the human. It is recommended that future studies focus initially on determining these kinetic parameters in the rat in order to complete a PBPK model in this species, followed by analogous studies in the primate. Primate studies are needed because it has been established that the toxic metabolite of methanol, formate, does not accumulate in the rat following methanol poisoning but it does in the human and nonhuman primate (Tephly and McMartin, 1984; HEI, 1987).

INTRODUCTION

The extensive use of gasoline and diesel fuel in our society has resulted in an increase of potentially toxic and carcinogenic pollutants (ozone, particulate matter, benzene, nitrogen oxides, etc.) in the ambient air. Methanol has been proposed as a replacement fuel for vehicles due to its generally cleaner combustion properties (ARB, 1986). However, a potential public health risk exists regarding the possible repeated exposure of individuals to methanol that may be spilled onto the skin during fueling. Methanol, through its metabolite formate, can be very toxic, producing such symptoms as central nervous system depression, nausea, and visual disturbances at low exposures, and metabolic acidosis, blindness, and coma at high exposure levels (Tephly and McMartin, 1984; HEI, 1987).

That methanol exposure via the dermal exposure route warrants special consideration is indicated by the fact that methanol, unlike all other alcohols and most organic solvents, is particularly damaging to the skin, increasing skin permeability severalfold via delipidization of the stratum corneum (Scheuplein and Blank, 1971 and 1973). This effect is especially important at high methanol concentrations. Thus, methanol can facilitate its own absorption via the induction of significant skin damage. In addition, there is some evidence which suggests that the dermal absorption of methanol may be enhanced by gasoline in methanol/gasoline fuel mixtures (Ferry et al., 1982). The enhanced methanol absorption observed in methanol/gasoline mixtures may be due to additional skin damage produced by the gasoline component. Ferry et al. (1982) reported greater skin damage on the hands of individuals treated with methanol and gasoline compared to those treated with pure methanol.

Currently available information regarding the dermal absorption of methanol is limited primarily to in vitro studies. Gummer and Maibach (1986) studied the penetration of methanol through excised, full-thickness, guinea pig skin. They found that only 1% of the total dose penetrated the skin after 19 hours. In vitro dermal absorption rates are frequently expressed as a permeability coefficient, K_p (units of cm/h). K_p derives from Fick's law and is calculated by dividing the absorption rate ($\mu g/(cm^2\text{-}h)$) by the applied concentration of penetrant ($\mu g/cm^3$). The

larger the value of K_p for a chemical, the more readily the chemical is absorbed. Theoretically, K_p is independent of the penetrant concentration but dependent on the vehicle (Scott and Dugard, 1989). Durrheim et al. (1980) measured a dermal permeability coefficient for methanol of 2.6 $\times 10^{-3}$ cm/h using a diffusion cell system and full-thickness, hairless mouse skin (the abdominal skin of the hairless mouse strain HRS/J). Delterzo et al. (1986) reported a methanol permeability coefficient of 4.4×10^{-3} cm/h in excised nude rat skin. Scheuplein and Blank (1973) determined the K_p for both pure methanol and methanol in aqueous solution (0.1 M) using human stratum corneum in a diffusion cell. The K_p for pure methanol (10.4×10^{-3} cm/h) was about 20 times higher than that for methanol in aqueous solution (0.5×10^{-3} cm/h). Evidence was presented indicating that the much higher absorption of pure methanol was due to the damaging effect of pure methanol on the integrity of the stratum corneum. Treherne (1956) measured a K_p for methanol of about 2.5×10^{-3} cm/h in whole rabbit skin. This value was constant for several different concentrations of methanol in aqueous solution. When just the dermis was used, a K_p of 1.2×10^{-3} cm/h was obtained, indicating that the stratum corneum provides the primary resistance layer to absorption.

An extensive search of the literature revealed only one in vivo study of dermal methanol absorption. In this study, Dutkiewicz (1980) measured a methanol dermal absorption rate of 11,400 ug/(cm^2-h) corresponding to a K_p of 1.4×10^{-2} cm/h in human volunteers. This rate was based on the application of pure methanol to the forearm with complete occlusion to prevent evaporation.

The lack of quantitative in vivo data on the dermal absorption of methanol constitutes an important data gap for assessing methanol health risks. In vivo systems offer several advantages over in vitro systems, including most importantly, the presence of a completely viable and intact skin barrier. In addition, skin temperature effects on absorption are likely to be more realistic in in vivo systems, particularly as the increased in vivo skin temperature may increase the evaporation rate of a volatile penetrant. Finally, the presence of an intact skin capillary network to serve as a continually-exchanged sink for the penetrant may have an important effect on absorption kinetics compared to the stagnant receptor fluid in a diffusion cell.

PROJECT OBJECTIVES

The objectives of the project were:

1) Quantitative measurements of the rates of methanol uptake by the skin following the dermal application of methanol alone or when present with gasoline in mixtures at five different concentrations.

(2) Quantitative measurements of tissue uptake and retention of methanol in brain regions, lung, liver, kidney, blood, and carcass during 24 h following the dermal application of methanol alone or when present with gasoline in methanol/gasoline mixtures.

(3) Measurements of elimination half-life of methanol and metabolites (as ^{14}C-equivalents) in major tissues and in the whole body.

(4) Measurements of the fraction of dermally-absorbed methanol excreted in the urine, feces, and exhaled as parent compound and/or metabolites (as ^{14}C-equivalents) as observed during 24 h following treatment.

(5) Measurements of the fraction of applied dose that evaporates from the treatment sites as observed during 24 h post-treatment.

6) Development of a preliminary physiologically-based pharmacokinetic model of methanol disposition in the rat following dermal application of methanol.

METHODS

Chemicals

Radiolabeled ^{14}C-methanol (specific activity = 4.1 mCi/mmol) was obtained from New England Nuclear (Boston). Solvents and other chemicals were purchased from J.T. Baker Chemical Co., Scientific Products, Packard, Inc., and other reputable suppliers. All reagents used were reagent grade. Unleaded gasoline (SU 2000) was purchased from a Shell gas station (Davis, CA) on June 14, 1990 and stored under nitrogen at 4°C during the study. A sample of the gasoline was sent to Coast-to-Coast Analytical Services, Inc. (San Luis Obispo, CA) before and after the study (on 7/27/1990 and 9/19/1990) to determine whether any significant changes in the gasoline components occurred over the study period. However, due to a misunderstanding the wrong anaylsis was performed on the sample and no data regarding the relative fractions of the various alkane fractions are available.

Animal Treatment

Overall, a total of 120 young adult, specific pathogen-free (SPF), male Sprague-Dawley rats were used in this project (104 experimental and 16 in a pilot study). The animals were purchased from Bantin and Kingman (Fremont, CA) and were shipped in SPF-filtered containers. Upon arrival, the animals were housed in stainless steel cages at the ITEH animal housing facilities and provided water and Purina rat chow ad libitum. Prior to treatment, the animals were ear-tagged with an identification number, weighed, and the hair on their backs clipped using an electrical clipper. A total of 96 rats were treated dermally with 0.5 mL of either pure methanol or one of five methanol/gasoline mixtures containing 95, 90, 85, 50 or 5% methanol. The solution was topically applied to the preclipped skin using a digital microliter pipet (Pipetman, Model P-200D, West Coast Scientific, Hayward, CA). The estimated size of the treated area was 25 cm^2. After treatment, a Queen Anne collar made of polyethylene sheeting (1.0 mm in thickness, 11.25 cm in diameter) with a neck opening of 2.5 cm in diameter, was placed around the neck of each animal and fastened to prevent the animals from grooming the

treated areas. An additional 24 rats were injected subcutaneously with 50 µL of pure methanol or a methanol/gasoline mixture to allow determination of the fraction of absorbed methanol exhaled as methanol vapor and carbon dioxide. These data are presented in Appendix D. A summary of the experimental design is provided in Appendix A, Table A-1.

The original plan involved the use of a special skin depot device (mounted on the backs of the rats) for dermal treatment and methanol vapor collection. The rats would then be placed in standard steel rodent cages and the vapors collected from the skin depot device. Initial studies of this approach, however, revealed that the use of the skin depot device virtually eliminates normal evaporation of methanol from the skin surface. Use of this device would therefore result in an artificially high estimate of dermal absorption since it would completely eliminate the competing loss process of evaporation. A typical human exposure to methanol during auto fueling would probably have no occlusion of the skin following the spill. Therefore, the skin depot device was not used in order to better simulate a typical human exposure scenario. Instead, the rats were housed in flow-through glass metabolism chambers which allowed for complete collection of naturally evaporated methanol vapors from the unoccluded skin surface. It also allowed separate collection of absorbed methanol that was metabolized to carbon dioxide. A schematic diagram of the metabolism chamber system is shown in Appendix B. The number of rats per group was changed to accommodate this modification of the original experimental design. The use of the glass metabolism chamber greatly increased the work associated with each rat, but also increased the completeness of the data collected.

The animal experimentation sections of this project were carried out in accordance with the principles of the American Association for Accreditation of Laboratory Animal Care (AAALAC) and the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH, 1985). The protocol for the use of animals in this project was approved (approval #4157, on 4/10/90) by the Campus Veterinarian and the Animal Research Use Administrative Advisory Committee at the University of California, Davis.

Sample Collection

Immediately following treatment, the rats were housed individually in all-glass metabolism cages (Vanguard International, Inc., Neptune, NJ) with food and water provided ad libitum. The excreta, exhaled and evaporated methanol vapor, and carbon dioxide were collected over the subsequent 24 h period. Samples were not collected at 48 h because ^{14}C-methanol equiv. concentrations declined to background levels at this time. Methanol vapors exiting the metabolism chambers were trapped in two water bubblers placed in series. Carbon dioxide was trapped in a third bubbler containing 2 M KOH. An illustration of the test system is presented in Appendix B, Figure B-1. Urine and fecal samples were collected from the rats at 1, 4 and 24 h, and placed in preweighed and prelabeled containers. For the pure methanol application, additional biological samples were also collected at 0.5 h. The cages were decontaminated daily after the collection of the excreta with 2% Isoclean solution and water. The cage washes were collected and analyzed for ^{14}C. During the collection, the appropriate radiological safety precautions were followed as described in a protocol approved by the U.C. Davis Office of Environmental Health and Safety.

Necropsy

The animals were sacrificed in groups of four at each time point. Treated rats were sacrificed via overdose of sodium pentothal (70 mg/kg ip) and exsanguinated via cardiac puncture. Tissue samples taken from each animal included brain, liver, lungs, fat, kidneys, and skin at the site of application. Upon removal, all tissues were examined grossly by a veterinary pathologist. All tissues appeared normal. Skin at the site of application was removed and washed with 100 mL of double-distilled water to collect any residual ^{14}C-methanol remaining on the skin surface. All tissues were weighed and immediately stored frozen at -20°C until analysis.

Sample Preparation for Radioanalysis

Carcass, GI tract with contents, and fecal samples were ground with dry ice using a Thomas Wiley Mill tissue grinder Model #E0-5. The resulting powders were placed in pre-weighed aluminum foil boats and kept at room temperature for one to two days to evaporate all added dry ice. The powders were then combusted using a Harvey Biological Material Oxidizer (R.J. Harvey Instrument Company, Hillsdale, NJ). Three preweighed aliquots from each sample (60-100 mg) were combusted. The combustion efficiency for methanol was determined using the appropriate ^{14}C standard. Carbon 14-labeled carbon dioxide produced during combustion was collected in an alkylamine scintillation cocktail (R.J. Harvey Corp., NJ).

Brain and lung samples were digested individually with protosol as follows:
1) protosol was added to each tissue (6 mL per gram) and the vial tightly capped and placed in a 55°C bath for 24 hours; 2) the vials were removed from the water bath, allowed to reach room temperature and the contents frozen; 3) 0.1 mL of 30% H_2O_2 was then added to each vial, the vials tightly capped, and heated at 55°C for 30 minutes; 4) the vials were allowed to come to room temperature again and the contents frozen. Prior to scintillation counting the samples were thawed and a 10 mL aliquot of scintillation cocktail was added to each vial. Liver, kidney, and fat tissues were combusted directly to $^{14}CO_2$ as described above for the carcass. Blood was centrifuged for 10 minutes at 3000 RPM using a clinical centrifuge to separate red blood cells from plasma. Urine, skin wash, bubbler water, KOH and plasma were counted directly.

Radioanalysis

Combusted samples were counted directly in an alkylamine scintillation fluid following a 24 h equilibration period. For all other samples a 10 ml aliquot of 3a70B scintillation fluid was added to the sample vial followed by a 24 h equilibration period. Samples were counted using a Packard Tri-Carb 300C preprogrammed liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) calibrated for ^{14}C with NBS standards. Quench corrections were made utilizing 3a70B scintillation cocktail (Complete Counting Liquid 3a70B, Research Products

International Corp., Mount Prospect, IL). Each sample was counted for 10 minutes or sufficiently long to achieve a statistical coefficient of variation of 0.5% over a beta particle energy region of 0-156 KeV. A 10 minute count of typical 50 dpm background yielded a coefficient of variation of about 5%. Analyses of control tissues from unexposed rats were used to evaluate background levels.

Estimation of Methanol Dermal Absorption Efficiency

The methanol dermal absorption efficiency (DAE) for each methanol/gasoline mixture was calculated as follows:

$$DAE = \frac{AA-AEV-ASKW}{AA} \times 100 \qquad (1)$$

where AA is the amount of methanol applied (mmol), AEV is the amount of methanol evaporated (mmol), and ASKW is the amount of methanol removed from the application site via the skin wash.

Statistical Analysis

The mean estimated dermal absorption efficiency of methanol for each methanol/gasoline fuel mixture was compared statistically to that for pure methanol using several procedures. First, Bartlett's test was used to assess equality of variances between all of the means. Next, pairwise F tests (between each mixture and pure methanol) were conducted to identify pairs with unequal variances. Finally, those pairs of means demonstrating unequal variances were compared using Welch's t-test (Shott, 1990). Mean pairs with equal variances were compared using Student's t-test. All t-tests were carried out using the SYSTAT statistical software (Wilkinson, 1990).

Physiologically-Based Pharmacokinetic (PBPK) Model Development

A PBPK model was developed to simulate the fate of methanol when applied to the skin of the rat. A schematic of the model is presented in Figure 1. A detailed description of the model equations and assumptions is presented in Appendix C. The basic structure of the model is similar to that originally developed by Ramsey and Anderson (1984). Thus, compartments for the liver, fat, rapidly perfused tissue (viscera) (RPT) and slowly perfused tissue (muscle and skin) (SPT) were included. However, the Ramsey and Anderson model was supplemented with additional compartments to represent the dermal dosing site, the arterial blood and venous blood, and the metabolism chamber. The metabolism chamber was explicitly included in the model because the rate of appearance of methanol and carbon dioxide in the collection apparatus is a function of the volume of the metabolism chamber and the air flow rate through it. Loss from the dermal dosing site was assumed to occur via simultaneous evaporation and dermal absorption of methanol. Elimination was assumed to occur primarily via liver metabolism of methanol directly to carbon dioxide. This represents a considerable simplification of actual methanol metabolism (Figure 2), however, it is justified at the low methanol dose levels used here and in this particular species due to the extremely short half-lives of the intermediate metabolic species in the rat. Elimination is also expected to occur via respiratory and urinary elimination of methanol. Since methanol was administered dermally and not orally, fecal elimination was modeled as the more relevant mechanistic process of biliary excretion. Although biliary excretion of a toxicant metabolite is normally significant only for high molecular weight compounds (Klaasen, 1986), some biliary excretion can also be expected to occur for any compound which distributes into the total body water. Methanol distributes to the total body water and it has been found in the bile of dogs (Yant and Schrenk, 1937).

Figure 1

Physiologically-Based Pharmacokinetic Model for Methanol

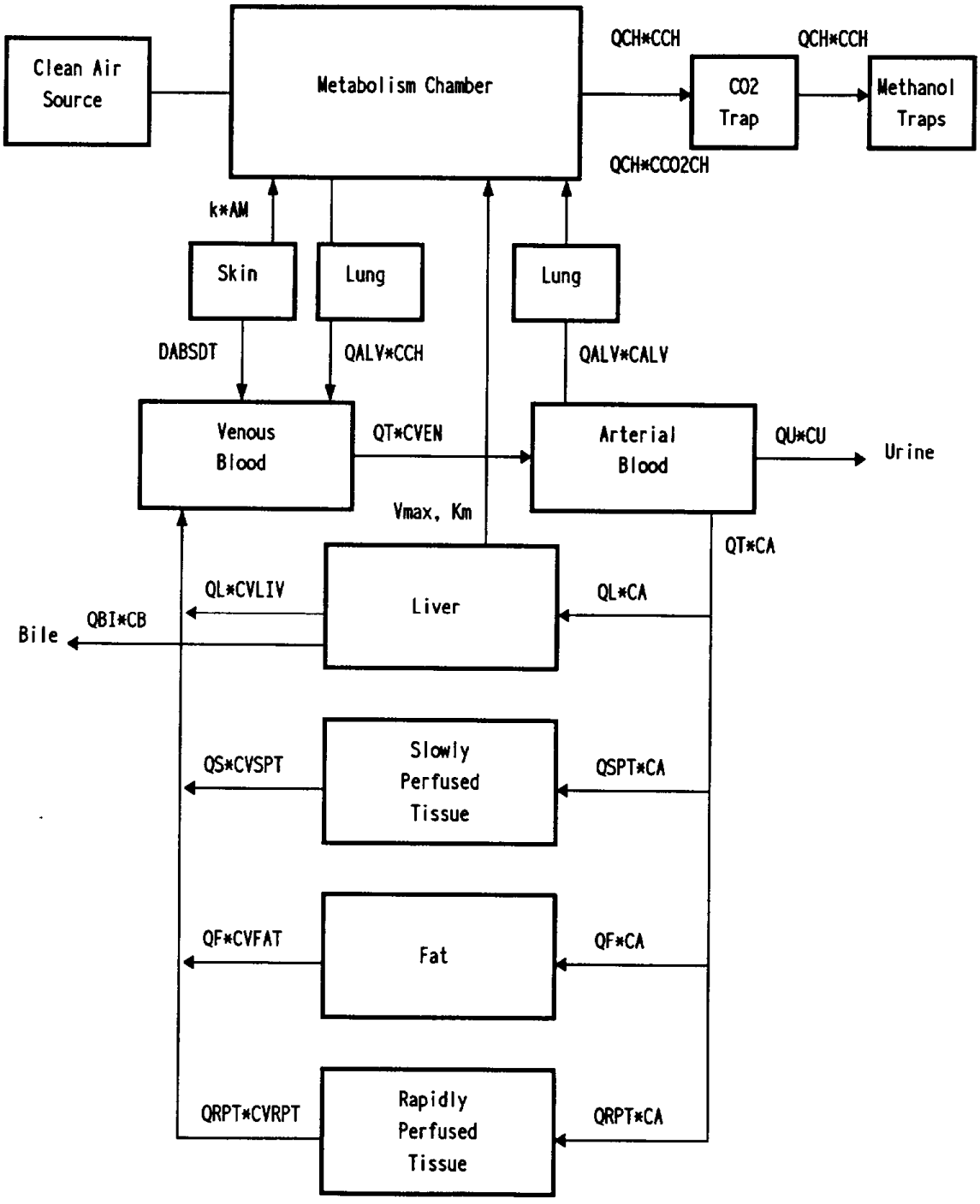
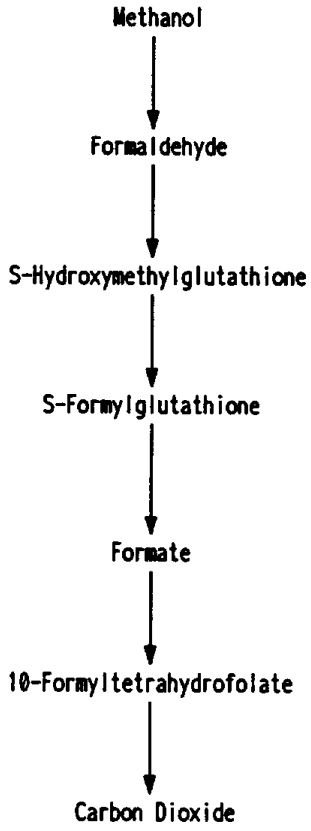


Figure 2

Simplified Schematic of Methanol Metabolism



RESULTS

Dermal Absorption of Methanol

Specific time-based rates of methanol absorption (e.g. mmol/(cm^2-h) or μg/(cm^2-h)) could not be determined due to the very rapid absorption kinetics of methanol (complete absorption occurred before any experminental data were collected). However, the effect of gasoline concentration on the efficiency with which methanol is absorbed from methanol/gasoline fuel mixtures was determined. Estimates of the dermal absorption efficiency (DAE) of methanol for pure methanol and each of the tested fuel mixtures are shown in Table 1. Only the values for the 5% and 50% methanol mixtures were found to be statistically significantly different (greater) than that for the 100% methanol treatment ($p<0.05$)). Thus, gasoline concentrations equal to or greater than 50% appear to enhance the dermal absorption of methanol from methanol/gasoline fuel mixtures.

Although methanol absorption appears to be enhanced from high gasoline concentration mixtures, a measure of dermal absorption more relevant to human health risk assessment is the amount of methanol absorbed per cm^2 of wetted skin surface area. It is this parameter which determines the total amount of methanol likely to be absorbed systemically following an accidental spill onto the skin. These values for pure methanol and each of the mixtures are shown in Table 1. It is apparent that even though the DAE of methanol increases significantly at gasoline concentrations of 50% or more, the much lower concentrations of methanol in the high percent gasoline mixtures results in a much smaller total amount of methanol being absorbed per cm^2 of wetted skin surface.

Fate of Methanol Following Dermal Application

Evaporation was the most important fate process for dermally-applied methanol. About 61-79% of the applied methanol evaporated from treatments consisting of 100, 95, 90, and 85% methanol (Tables 2-5). Evaporative losses were reduced at higher gasoline concentrations.

Table 1

Dermal Absorption of Methanol from Methanol/Gasoline Fuel Mixtures

	Percent Methanol in Methanol/Gasoline Fuel Mixture					
	100	95	90	85	50	5
Methanol content in 0.5 ml applied fuel mixture (mmol)	11.9	11.3	10.8	10.2	6	0.6
Percent of applied methanol dermally absorbed[1]	24.8 (3.7)	31.6 (4.5)	24.5 (1.4)	27.0 (2.9)	35.7 (1.8)[2]	58.1 (4.2)[2]
umol methanol absorbed[1] per cm^2	120 (17)	140 (20)	110 (6)	110 (12)	86 (4)[2]	14 (1)[2]

[1]Values are means and (standard errors of the means) of 12 animals.

[2]Significantly different from 100% methanol treatment ($p<0.05$)). See statistical analysis in Methods.

Table 2

Fate of C-14 Methanol Following Dermal Application in the Rat

(100% Methanol)

Percent of Applied Dose	Percent of C-14 Methanol at Times Postdosing			
	0.5 h	1 h	4 h	24 h
Evaporated	78.2 (10.2)	72.7 (19.8)	78.9 (7.7)	74.1 (10.9)
Remaining at application site	0.1 (0.0)	ND	ND	ND
Dermally absorbed	21.8 (10.2)	27.3 (19.8)	21.1 (7.7)	25.9 (10.9)
Percent of Dermally-Absorbed Dose				
Total body (all tissues, muscle, bone, skin, blood)	89.8 (14.0)	98.3 (0.8)	67.6 (26.6)	44.1 (9.8)
Selected tissues				
Brain	0.4 (0.2)	0.2 (0.2)	0.3 (0.1)	0.1 (0.0)
Liver	0.3 (0.1)	0.4 (0.3)	0.7 (0.4)	0.6 (0.1)
Lungs	0.4 (0.2)	0.2 (0.2)	0.3 (0.1)	0.2 (0.0)
Kidney	0.3 (0.1)	0.4 (0.4)	0.6 (0.3)	0.4 (0.0)
Exhaled as CO_2	6.8 (9.3)	0.9 (0.3)	25.4 (22.4)	43.4 (9.3)
Exhaled as methanol	3.4 (4.7)	0.4 (0.1)	4.6 (4.0)	4.9 (1.1)
Urine	No sample	0.3 (0.3)	1.6 (0.5)	7.0 (3.9)
Feces	No sample	0.1 (.2)	0.8 (0.7)	0.7 (1.2)

Values are means and standard deviations of four animals.
ND = Not detected (less than 0.05%).

Table 3

Fate of C-14 Methanol Following Dermal Application in the Rat

(95% Methanol)

Percent of Applied Dose	Percent C-14 Methanol at Times Postdosing 1 h	4 h	24 h
Evaporated	72.2 (17.1)	61.0 (22.0)	72.0 (4.4)
Remaining at application site	0.1 (0.0)	ND	ND
Dermally absorbed	27.8 (17.1)	39.0 (22.0)	28.0 (4.4)
Percent of Dermally-Absorbed Dose			
Total body (all tissues, muscle, bone, skin, blood)	91.2 (10.1)	82.9 (12.6)	68.0 (4.1)
Selected tissues			
Brain	0.3 (0.1)	0.2 (0.1)	0.1 (0.0)
Liver	0.3 (0.1)	0.5 (0.3)	0.5 (0.1)
Lungs	0.3 (0.1)	0.2 (0.1)	0.1 (0.0)
Kidney	0.3 (0.1)	0.4 (0.3)	0.3 (0.1)
Exhaled as CO_2	7.5 (8.8)	11.2 (6.3)	24.4 (4.4)
Exhaled as methanol	1.0 (1.2)	1.5 (0.9)	3.3 (0.6)
Urine	0.2 (0.2)	4.2 (6.0)	3.3 (1.2)
Feces	0.1 (0.1)	0.2 (0.1)	1.0 (0.6)

Values are means and (standard deviations) of four animals.
ND = Not detected (less than 0.05%).

Table 4

Fate of C-14 Methanol Following Dermal Application in the Rat

(90% Methanol)

Percent of Applied Dose	Percent C-14 Methanol at Times Postdosing 1 h	4 h	24 h
Evaporated	74.5 (4.2)	76.5 (7.5)	75.6 (3.1)
Remaining at application site	0.1 (0.0)	0.1 (0.0)	ND
Dermally absorbed	25.5 (4.2)	23.5 (7.5)	24.4 (3.1)
Percent of Dermally-Absorbed Dose			
Total body (all tissues, muscle, bone, skin, blood)	94.3 (2.0)	74.8 (12.8)	57.2 (1.9)
Selected tissues			
Brain	0.4 (0.1)	0.4 (0.1)	0.1 (0.0)
Liver	0.9 (0.2)	1.2 (0.6)	0.8 (0.1)
Lungs	0.3 (0.1)	0.3 (0.1)	0.1 (0.0)
Kidney	1.1 (0.3)	1.0 (0.5)	0.6 (0.1)
Exhaled as CO_2	4.8 (1.6)	18.0 (8.9)	30.4 (1.8)
Exhaled as methanol	0.8 (0.3)	2.9 (1.4)	4.9 (0.3)
Urine	0.2 (0.1)	3.9 (4.4)	5.5 (1.5)
Feces	No sample	0.4 (0.2)	2.1 (0.9)

Values are means and (standard deviations) of four animals.
ND = Not detected (less than 0.05%).

Table 5

Fate of C-14 Methanol Following Dermal Application in the Rat

(85% Methanol)

Percent of Applied Dose	Percent C-14 Methanol at Times Postdosing		
	1 h	4 h	24 h
Evaporated	76.3 (6.6)	78.6 (7.9)	64.1 (10.5)
Remaining at application site	0.3 (0.1)	0.2 (0.0)	ND
Dermally absorbed	23.7 (6.6)	21.4 (7.9)	35.9 (10.5)
Percent of Dermally-Absorbed Dose			
Total body (all tissues, muscle, bone, skin, blood)	91.8 (2.9)	67.4 (14.0)	62.2 (8.8)
Selected tissues			
Brain	0.4 (0.1)	0.4 (0.1)	0.1 (0.0)
Liver	0.7 (0.2)	1.0 (0.3)	0.6 (0.2)
Lungs	0.2 (0.1)	0.3 (0.1)	ND
Kidney	0.7 (0.2)	0.9 (0.3)	0.5 (0.1)
Exhaled as CO_2	6.0 (1.8)	22.7 (9.6)	25.3 (6.5)
Exhaled as methanol	1.0 (0.3)	3.8 (1.6)	4.2 (1.1)
Urine	1.1 (0.8)	5.3 (3.2)	6.3 (2.3)
Feces	ND	0.8 (0.8)	2.1 (0.9)

Values are means and (standard deviations) of four animals.
ND = Not detected (less than 0.05%).

About 64% of the applied methanol evaporated from the 50% mixture (Table 6) and only 30-48% of the applied methanol evaporated from the 5% mixture (Table 7). Evaporation occurred rapidly, being complete within 30 min following application of 100% methanol (Table 2).

Of the fraction of methanol dermally absorbed, only a small portion (<2%) could be accounted for in the major tissues (brain, liver, lungs, and kidneys) at any time up to 24 h post-dosing (Tables 2-7). Since most of the absorbed methanol is present in the body at early time points (Tables 2-7), it is probable that the majority of absorbed methanol distributed into the large bulk of muscle tissue. This is supported by mass balance calculations which indicated that the vast majority of ^{14}C-methanol equiv. is in the carcass (which is mostly muscle tissue). The calculation formula used is shown in Appendix E, Figure E-1. Carcass concentrations could not be measured directly because the analytical method used (grinding with dry ice followed by drying overnight) resulted in complete evaporative loss of methanol during sample processing.

For all methanol/gasoline treatments, about 24-43% of the dermally-absorbed methanol was eliminated via exhalation as carbon dioxide at 24 h post-dosing (Tables 2-7). About 1-5% was exhaled as methanol. An additional 4-11% of the absorbed methanol was eliminated in the excreta at 24 h. The remaining label (44-68%) remained in the body at 24 h based on the mass calculations described above.

For most tissues (brain, lungs, liver, and kidneys), concentrations of ^{14}C-methanol equiv., although subject to significant variance at some time points, were generally similar to plasma concentrations (Figures 3-7). However, fat concentrations were significantly lower than plasma concentrations (Figure 7) and no elimination phase (reduction in concentration over time) was observed in the kidney tissue concentration (Figure 5).

Half-times were estimated for the elimination of ^{14}C-methanol equiv. from the plasma, brain, liver, lungs, and whole body. By definition, calculation of a half-time requires the

Table 6

Fate of C-14 Methanol Following Dermal Application in the Rat

(50% Methanol)

	Percent C-14 Methanol at Times Postdosing		
Percent of Applied Dose	1 h	4 h	24 h
Evaporated	63.1 (9.0)	63.5 (4.1)	66.1 (5.9)
Remaining at application site	0.3 (0.0)	0.2 (0.0)	0.1 (0.0)
Dermally absorbed	36.9 (9.0)	36.5 (4.1)	33.9 (5.9)
Percent of Dermally-Absorbed Dose			
Total body (all tissues, muscle, bone, skin, blood)	95.5 (1.9)	77.8 (5.1)	56.6 (2.1)
Selected tissues			
Brain	0.2 (0.0)	0.2 (0.0)	0.1 (0.0)
Liver	0.5 (0.1)	0.8 (0.2)	0.7 (0.2)
Lungs	0.2 (0.0)	0.2 (0.0)	0.1 (0.0)
Kidney	0.5 (0.0)	0.7 (0.1)	0.5 (0.2)
Exhaled as CO_2	3.9 (1.6)	14.0 (3.2)	29.0 (3.9)
Exhaled as methanol	0.4 (0.2)	1.4 (0.3)	2.8 (0.4)
Urine	0.1 (0.1)	6.7 (4.0)	10.0 (4.1)
Feces	0.1 (0.2)	0.1 (0.1)	1.5 (0.4)

Values are means and (standard deviations) of four animals.
ND = Not detected (less than 0.05%).

Table 7

Fate of C-14 Methanol Following Dermal Application in the Rat

(5% Methanol)

Percent of Applied Dose	Percent C-14 Methanol at Times Postdosing		
	1 h	4 h	24 h
Evaporated	48.5 (13.3)	30.1 (7.3)	46.2 (18.3)
Remaining at application site	0.2 (0.0)	0.1 (0.0)	ND
Dermally absorbed	51.5 (13.3)	69.9 (7.3)	53.8 (18.3)
Percent of Dermally-Absorbed Dose			
Total body (all tissues, muscle, bone, skin, blood)	92.3 (4.2)	81.4 (5.6)	52.4 (21.5)
Selected tissues			
Brain	0.1 (0.1)	ND	ND
Liver	0.6 (0.6)	0.1 (0.1)	0.1 (0.1)
Lungs	0.2 (0.2)	0.1 (0.0)	0.1 (0.1)
Kidney	0.4 (0.3)	0.1 (0.0)	0.1 (0.1)
Exhaled as CO_2	6.2 (4.5)	13.8 (2.1)	37.1 (16.6)
Exhaled as methanol	0.2 (0.1)	0.6 (0.1)	1.3 (0.6)
Urine	1.1 (1.1)	3.9 (3.6)	6.2 (5.2)
Feces	No sample	No sample	1.8 (1.2)

Values are means and (standard deviations) of four animals.
ND = Not detected (less than 0.05%).

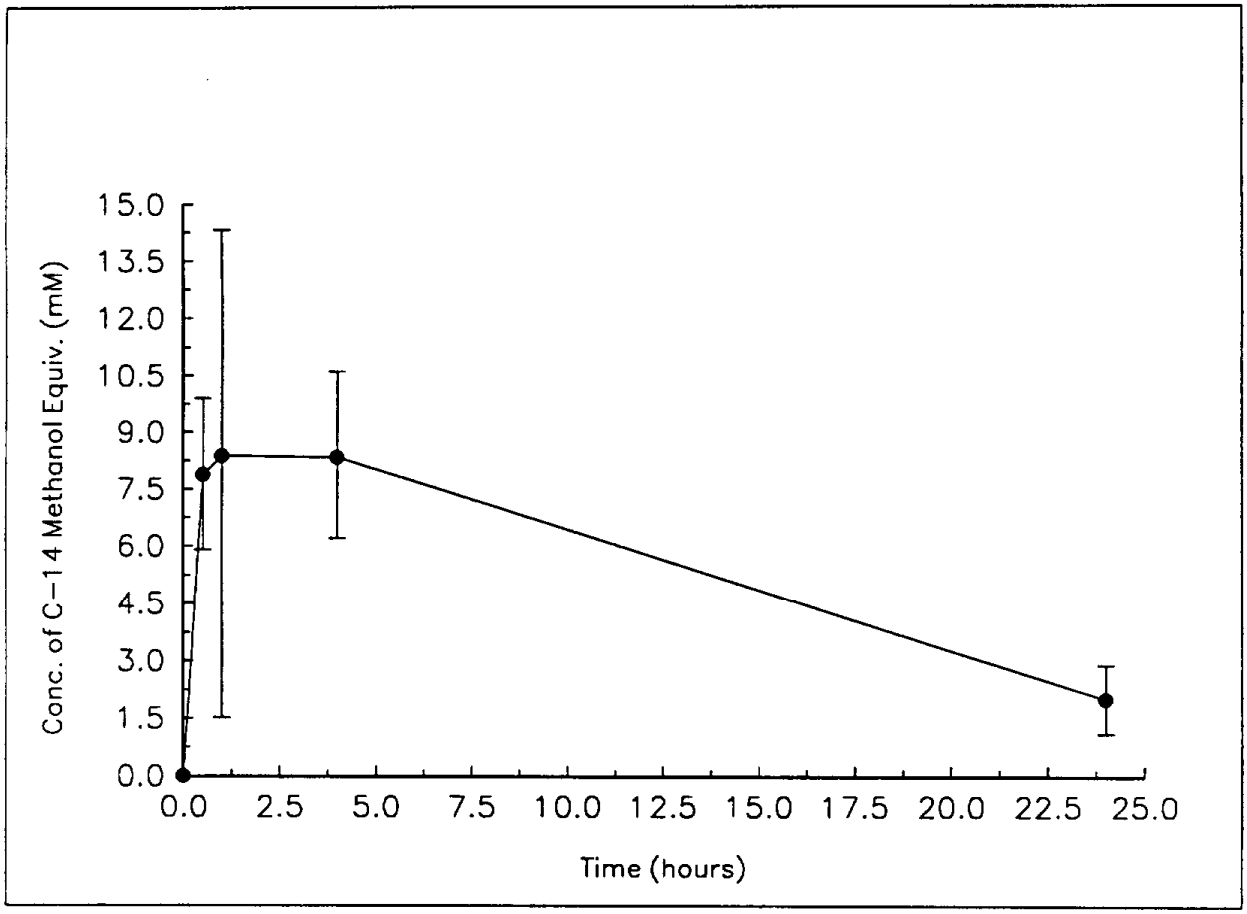


Fig. 3. Plasma concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

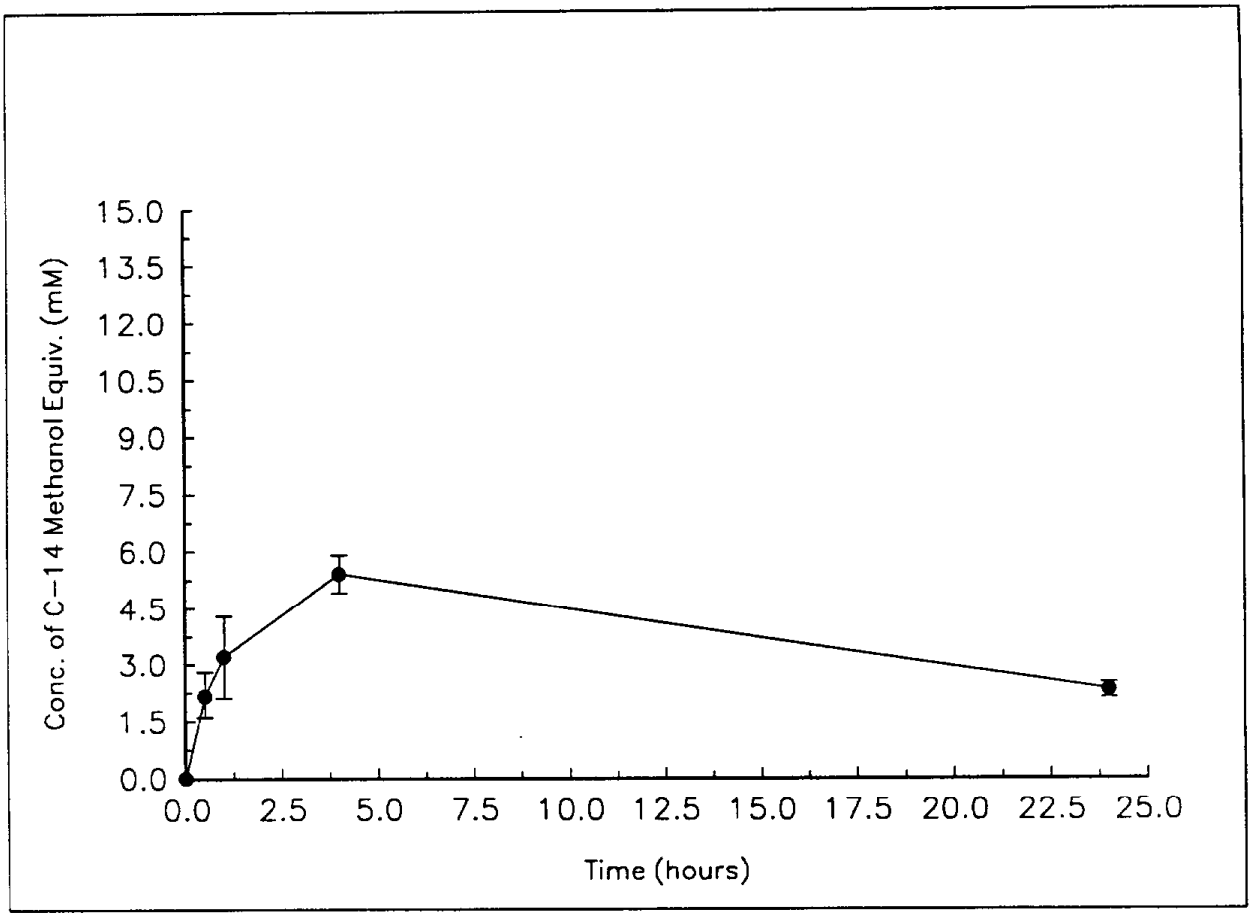


Fig. 4. Liver concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

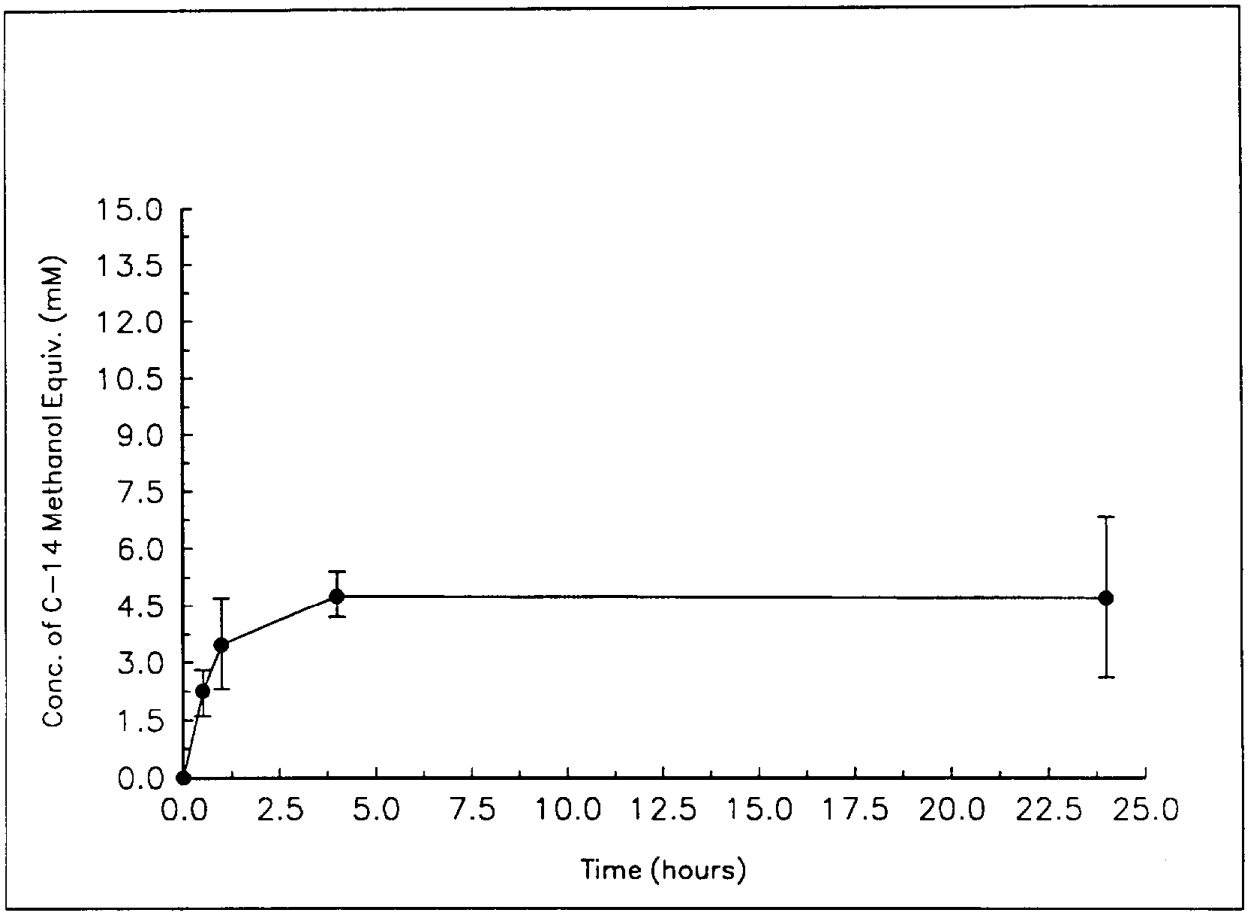


Fig. 5. Kidney concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

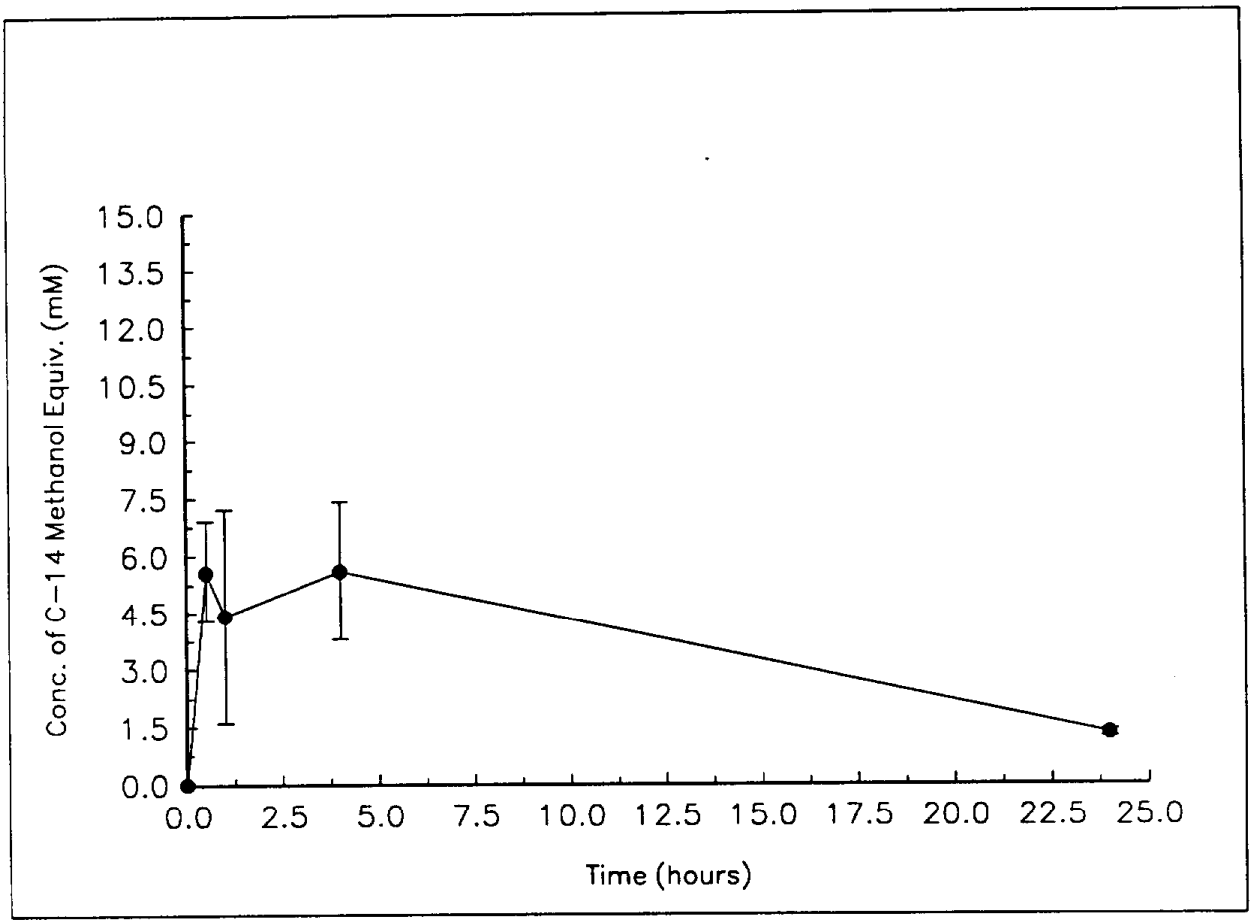


Fig. 6. Lung concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

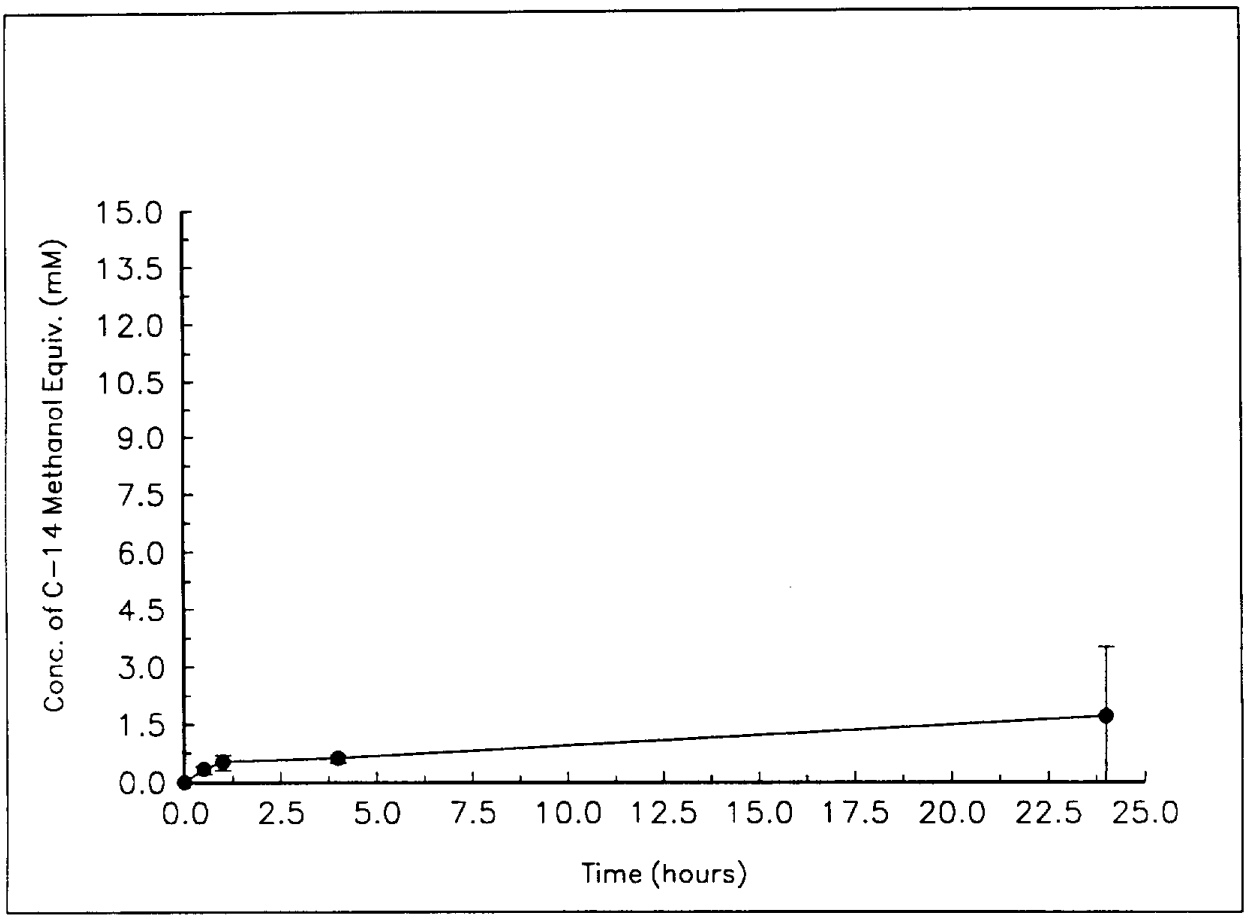


Fig. 7. Fat concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

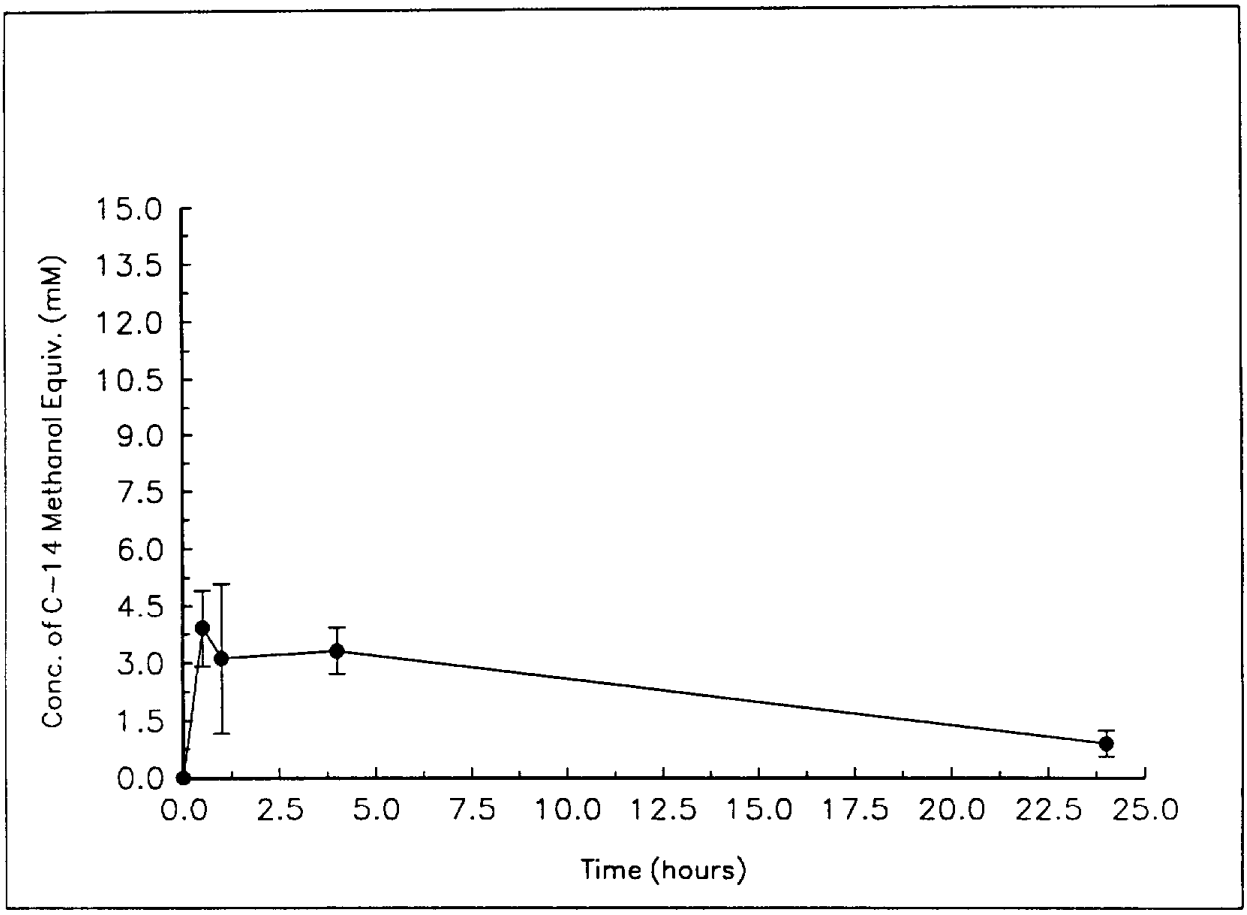


Fig. 8. Brain concentrations of ^{14}C-methanol equiv. following dermal application of 100% methanol in the rat. Values are means ±SD for four animals.

assumption that elimination is first-order. This assumption could not be validated in this study since there were insufficient data points collected to definitively characterize the kinetics of elimination (for example, to distinguish between Michaelis-Menten or first-order kinetics). The PBPK model, however, assumed Michaelis-Menten elimination kinetics because this is theoretically consistent with previously published data.

The half-times were found to be 9.7, 10.4, 16.3, 9.5, and 19.9 h for plasma, brain, liver, lungs, and whole body respectively. Half-times for fat and kidneys could not be determined because these tissues did not show any elimination during the 24 h.

PBPK Modeling

A physiologically-based pharmacokinetic model was developed to simulate the dermal absorption, tissue distribution, and elimination of topically-applied methanol in the intact rat. The model was fit to experimental data obtained for the 100% methanol treatment. However, all parameters used in the model, except the methanol evaporation rate constant (KEV) and the Michaelis-Menten constant (Km1) (used to model metabolic removal by the liver), were determined a priori from independent literature sources. These latter two parameters were determined by performing test simulations and adjusting the parameter values until a suitable fit was obtained. KEV was fit first, then assumed constant while Km1 was fit. This resulted in a KEV value of 7 per hr and a Km1 value of 20 mM. Because of the extremely rapid disappearance of methanol due to both evaporation and absorption, only one data point (at 0.5 h) was available for determining KEV. Thus, neither the quantitative value of this parameter nor the kinetics of this portion of the model (first-order vs. linear) could be effectively validated using the experimental data.

Overall model predictions of the fate of methanol in the in vivo rat model are illustrated in Figure 9. Due to the extreme rapidity of the evaporation and absorption process, only the first hour of the simulation is shown in Figure 9 in order to clearly illustrate the kinetics. Figure 9 shows the simulated amounts (mmol) of methanol remaining at the absorption site, evaporated,

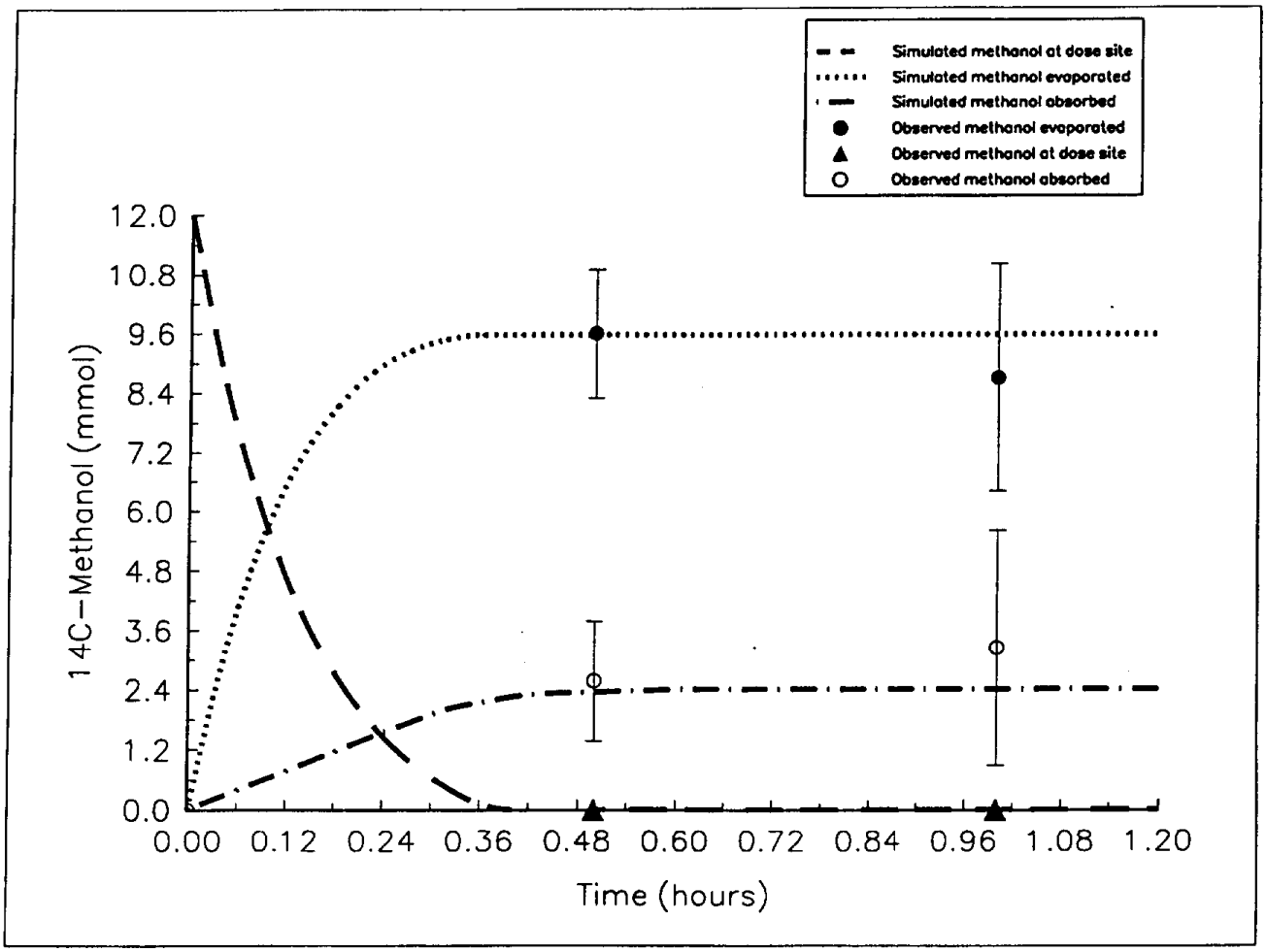


Fig. 9. Simulated and Observed Amounts of Methanol Evaporated, Dermally Absorbed, and Remaining at the Application Site. Values are means ± SD for four animals (no error bars are shown for the amount remaining at the site because the error was effectively zero).

and absorbed through the skin. Model predictions are consistent with the experimental data, although the lack of data during the first 30 min precluded complete validation of the kinetic assumptions of the model during this period. The model also predicts fairly well the plasma levels of methanol (Figure 10) and the amount of methanol converted to carbon dioxide (Figure 11). Model predictions of urinary and biliary excretion are within the range of the experimental error (Figure 12).

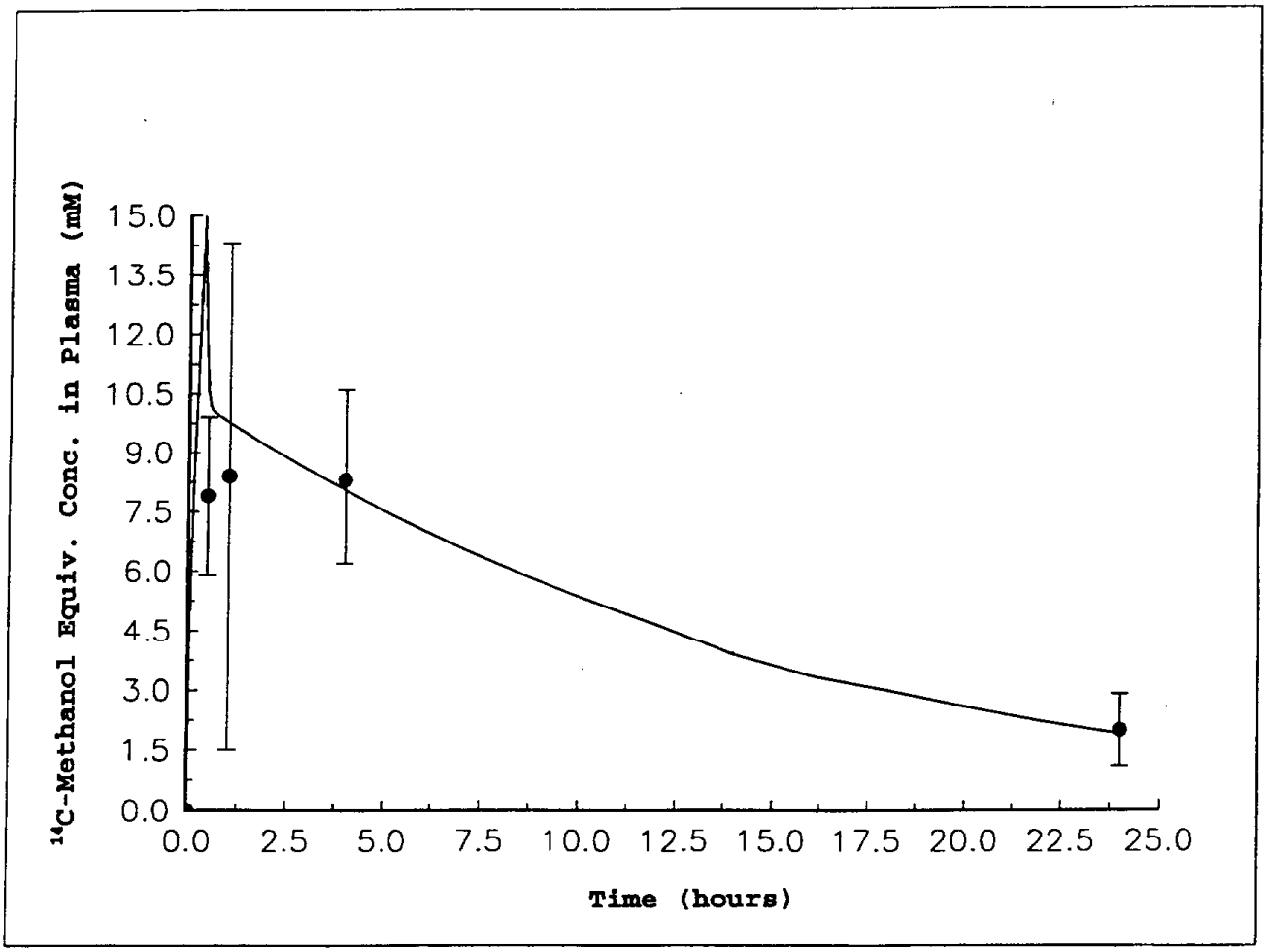


Fig. 10. Simulated and Observed Concentrations of ^{14}C-Methanol Equiv. in Plasma. Values are means ± SD for four animals.

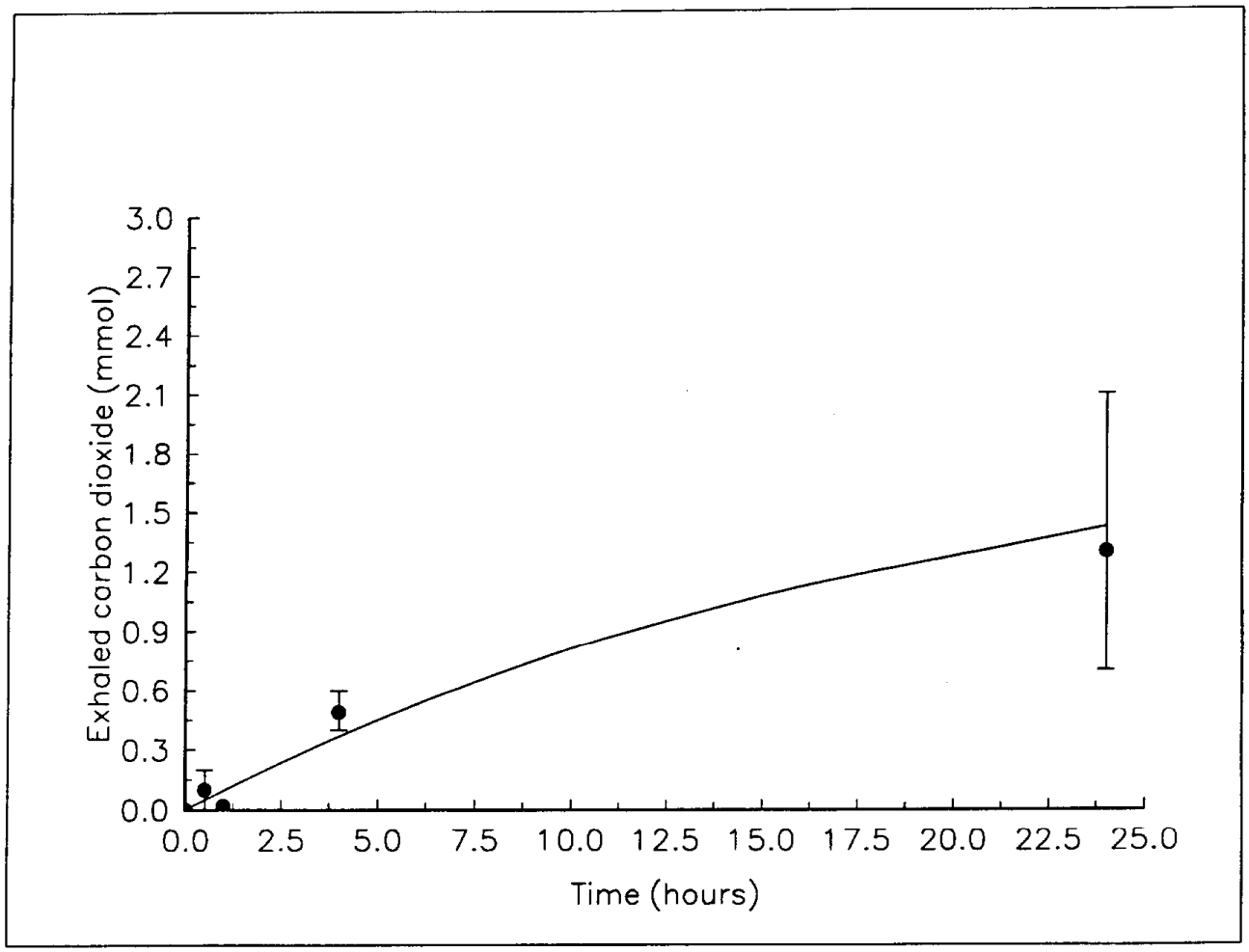


Fig. 11. Simulated and Observed Amounts of ^{14}C-Methanol Exhaled as Carbon Dioxide. Values are means ± SD for four animals.

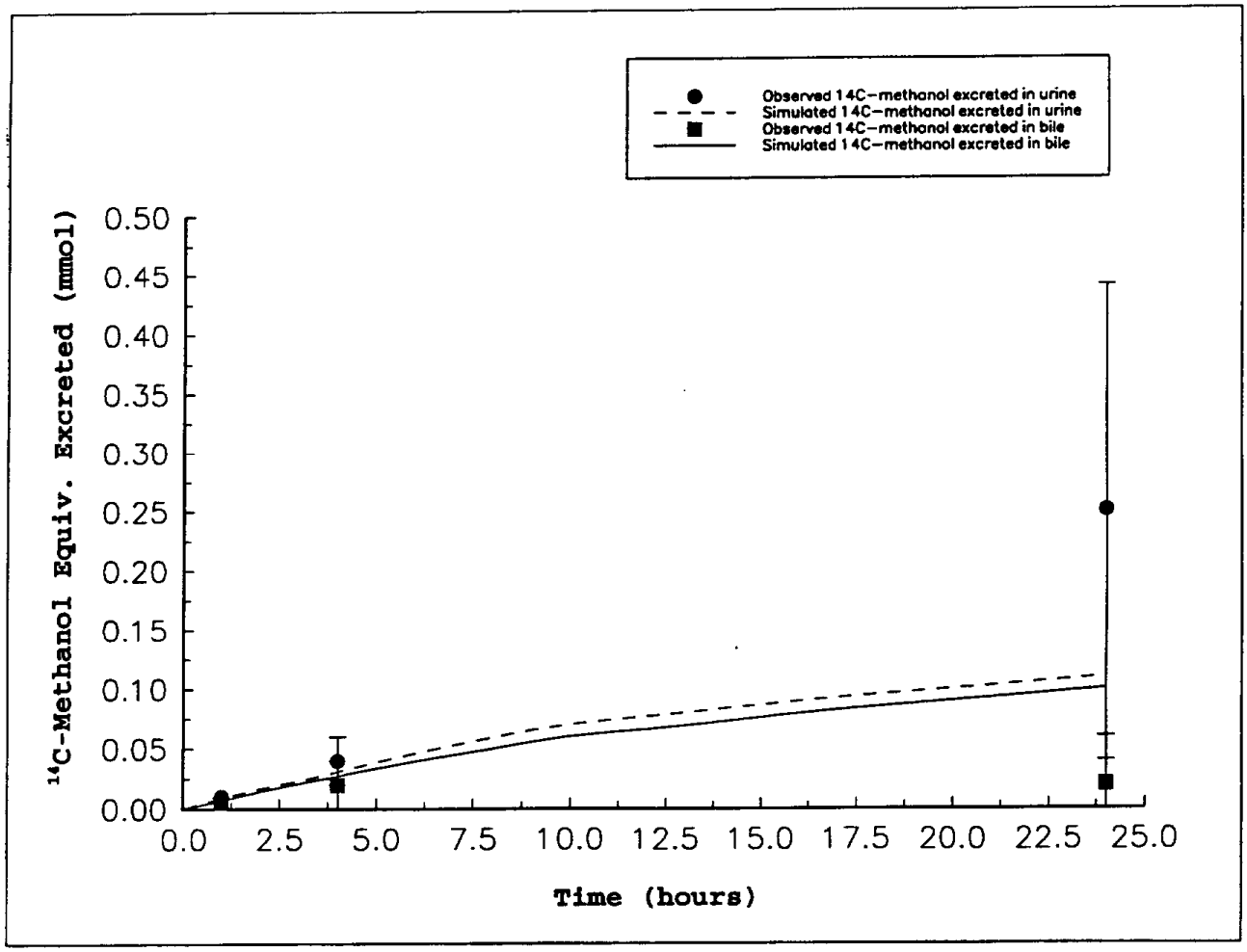


Fig. 12. Simulated and Observed Concentrations of ^{14}C-Methanol Equiv. Excreted in Urine and Bile. Values are means ± SD for four animals.

DISCUSSION

Dermal Absorption of Methanol

Dermal methanol absorption from methanol/gasoline mixtures was significantly enhanced relative to 100% methanol at gasoline concentrations of 50% or more. There are several possible explanations for this finding. First, the increased dilution of the applied methanol at high gasoline concentrations would reduce the methanol partial pressure since, by Henry's Law, the partial pressure of a compound in solution is proportional to its concentration. A reduction in the partial pressure of methanol would cause a reduction in the evaporation rate since the evaporation rate is directly proportional to the magnitude of the partial pressure gradient. This would in turn result in a longer residence time for the methanol on the skin and increased methanol availability for the competing fate process of absorption.

A second possibility is that gasoline may enhance methanol absorption by causing skin damage, with the effect being proportional to gasoline concentration. Ferry et al. (1982) observed greater skin damage on the hands of individuals exposed to both gasoline and methanol compared to pure methanol. The apparent increase in skin damage coincided with increased dermal absorption of methanol as measured by urine concentrations. Although gross pathological examination of the skin in the present study indicated no obvious skin damage, it is probable that significant damage could occur to the skin without being directly observable at the gross level.

A third explanation for the enhanced absorption of methanol at high gasoline concentrations is related to partitioning behavior. When a polar chemical is mixed with a nonpolar chemical the compounds will tend to separate into two distinct phases due to mutual repulsion. This is sometimes referred to as "salting out". In the case of the very nonpolar gasoline hydrocarbons, the very polar methanol will tend to be forced out of the applied solution. Since methanol is very soluble in the skin adjacent to the applied mixture (Scheuplein and Blank, 1971), the methanol will tend to leave the gasoline mixture and enter the skin. Furthermore, this

effect would be expected to be proportional to the gasoline concentration in the applied mixture. This latter explanation is supported by the observation that the very polar alcohol ethanol is absorbed three times more efficiently from a nonpolar mineral oil vehicle than when the alcohol is applied in water, a very polar vehicle (Blank, 1969). Conversely, the relatively nonpolar alcohol heptanol is poorly absorbed when applied in mineral oil compared to water. Thus, in general, maximal dermal absorption of a penetrant is observed when a vehicle is used which has a low solubility for the penetrant relative to the penetrant's solubility in the adjacent stratum corneum (Idson, 1983).

Although this study provided some evidence that the dermal absorption efficiency of methanol is enhanced at high gasoline concentrations, this is not the best measure for evaluating health risks due to human methanol exposures. A more relevant measure is the total amount of methanol absorbed per cm^2 of wetted skin surface. It is the total amount of methanol entering the systemic circulation which is more directly related to methanol toxicity. Thus, for example, even though the dermal absorption efficiency of methanol was enhanced at gasoline concentrations of 50% and 95%, the total amount of methanol absorbed was substantially reduced due simply to dilution (Table 1). Nonetheless, the dermal efficiency values obtained here are necessary values for the assessment of human health risks due to accidental fuel spillage on the skin. These values, within the limits of interspecies extrapolation, are essential parameters in calculating the likely systemic dose received from a spill.

In this study it was observed that absorption occurred very rapidly, being complete within at most 30 min and probably within 5-10 min. The extreme rapidity of the absorption process renders determination of a time-based "rate" of absorption largely superfluous. Multiplication of the dermal absorption efficiency values obtained here by the wetted area of the skin should provide a good approximation of the absorbed dose of methanol.

An important assumption of the present study is that dermal absorption data obtained in the rat are relevant to the human. The ability of the rat to quantitatively mimic dermal absorption in the human has been variable, depending on the chemical involved (Bartek et al.,

1972; Rougier et al., 1987). In the case of methanol, in vitro studies with excised skin indicate that the rat appears to overestimate the dermal absorption of methanol in the human. For example, several breeds of rat (nude, fuzzy, and furry) have demonstrated dermal permeabilities to methanol 3-9 times greater than that in humans (Delterzo et al., 1986). Specifically, the permeability coefficients (K_p) for the nude, fuzzy and furry rats were 4.4×10^{-3}, 1.8×10^{-3}, and 2.4×10^{-3} cm/h compared to a value for excised human skin of 0.5×10^{-3} cm/h. In addition, Scheuplein and Blank (1973) reported an even higher K_p value of 10.1×10^{-3} cm/h for pure methanol in excised rat skin which is 20 times higher than the corresponding human value.

No published studies reporting the dermal absorption of methanol in vivo in the rat could be located. Only one in vivo human study has been conducted which allows comparison to the in vivo rat results obtained here. Dutkiewicz et al. (1980) determined a K_p for pure methanol absorption of 1.2×10^{-3} cm/h in human volunteers. In this study the subjects' skin was occluded to prevent evaporation. Because absorption of methanol in the rat occurred so rapidly in the present study, an experimental estimate of the in vivo K_p could not be determined. However, the experimental results are in general consistent with the in vitro K_p used in the physiologically-based pharmacokinetic model. This value was 10.1×10^{-3} cm/h (Scheuplein and Blank, 1973). This suggests that the in vivo dermal absorption of methanol in the rat is likely to be at least 8-fold greater in the rat than in the human in vivo (assuming the upper limit K_p of 1.2×10^{-3} cm/h in the human) and is probably closer to 20 times greater (based on the in vitro human K_p of 0.5×10^{-3} cm/h). This conclusion is consistent with the general finding that rats tend to absorb a wide variety of chemicals through the skin more efficiently than humans (Bartek, et al. 1972).

The in vivo estimates of dermal uptake of methanol obtained here are also significantly higher than that reported by Gummer and Maibach (1986) in excised guinea pig skin. These investigators found that only 1% of the total dose penetrated the skin over a period of 19 hours. Possible reasons for the lower absorption through guinea pig skin in vitro are 1) the lack of an intact vascular system in the in vitro system resulting in the absence of a virtual "sink" for the absorbed methanol and 2) the lack of a blood supply to the excised skin may have resulted in a reduction of the vitality, integrity, and/or permeability of the skin.

Fate of Dermally Applied Methanol

The most important fate process for methanol following its topical application is evaporation. Evaporation accounted for 30-79% loss of the total methanol applied within 30 min. This was true for all mixtures studied, although the 50% and 5% methanol mixtures showed a reduced evaporation loss relative to 100% methanol. Evaporation thus constitutes an important competing fate process for methanol absorption, acting to dramatically reduce the amount of methanol available for absorption. Since the present studies were conducted in a fixed air flow metabolism cage, evaporation is likely to be even more important as a fate process in an outdoor setting where air movement is unrestricted and where temperatures may be higher at certain times of the year. Thus, total methanol absorption occurring under a typical fuel spill exposure scenario may be further reduced under some circumstances.

Concentrations of ^{14}C-methanol equiv. in liver, brain, lungs, and kidneys accounted for a very small fraction (total of less than 2%) of the absorbed methanol at all time points up to 24 h (Tables 2-7). Since mass-balance calculations indicated that the majority of the ^{14}C-methanol was still present in the body at the 1 and 4 h time points, most of the ^{14}C-methanol was probably distributed to the large bulk of muscle tissue. Methanol, like ethanol, is known to distribute evenly into the total body water (Yant and Schrenk, 1937; Haggard and Greenberg, 1939; Tephly and McMartin, 1984). Since most of the body tissues have a comparable water content, most of the ^{14}C-methanol would be present in the muscle based simply on the large mass of this tissue.

Concentrations of ^{14}C-methanol equiv. in the highly perfused tissues (liver, kidneys, lungs, and brain) were in the same range as plasma concentrations for all mixtures studied (Figures 3-8). This is consistent with the blood:tissue partition coefficient for methanol which is about one for highly perfused tissues (Yant and Schrenk, 1937; Fiserova-Bergerova and Diaz, 1986). However, no elimination phase was observed for the kidneys (Figure 5). This suggests possible concentration of ^{14}C-methanol equiv., probably as the metabolite formate, in this tissue. Formate is known to concentrate in the urine (McMartin et al., 1980; Tanaka et al., 1991). Fat concentrations were significantly lower than plasma concentrations which is consistent with the

low lipid solubility of methanol.

The elimination of ^{14}C-methanol equiv. was similar for all mixtures studied (including 100% methanol). About 25-40% of the dermally-absorbed ^{14}C-methanol was eliminated via metabolism and exhalation as carbon dioxide. An additional 1-5% was exhaled as methanol. About 1-5% of dermally-absorbed methanol was eliminated via the excreta (Tables 2-7). These values correspond well to results obtained in previous studies. Bartlett (1950), using rats, reported that at 48 h post-dosing, 60% of ingested ^{14}C-methanol was oxidized to carbon dioxide and 14% was exhaled as methanol. Urinary excretion of ^{14}C-methanol ceased after 24 h, totaling 3%. In monkeys, 32% of an oral dose was oxidized to carbon dioxide at 48 h postdosing (Noker et al., 1980). An additional 12% was excreted in the urine and 6% was exhaled as methanol. These results indicate that at the dose levels used in the present study, the general distribution and elimination of ^{14}C-methanol in the rat is a good model for methanol disposition in the primate.

PBPK Modeling

A physiologically-based pharmacokinetic model was developed to simulate the dermal absorption, distribution, and metabolic fate of methanol in the rat. Due to the limited number of time points at which samples were collected, a complete validation of the model was not possible. However, within the limitations of the data, the PBPK modeling was successful in simulating the major fate processes of methanol evaporation and absorption in the metabolism chamber system, as well as the overall elimination of absorbed methanol as carbon dioxide. In addition, the model accurately simulated concentrations of methanol in the blood and was reasonably consistent with urinary and biliary excretion data. However, in the case of elimination via the urine, large variance in the concentrations at each time point precludes conclusive validation (Figure 12).

The model demonstrates that evaporation of methanol is the most important fate process, both in terms of the amount of methanol being lost via this process and in terms of the rapidity

with which it occurs. Thus, evaporation acts as a critical competing process severely limiting the amount of methanol available for absorption. The extreme rapidity of the evaporation process was unexpected, since preliminary laboratory tests indicated a first-order evaporation constant for methanol of 0.18 per h. The final evaporation constant required to fit the experimental data was 7 per h. The increased evaporation of methanol in the metabolism chamber may be a function of several factors, including the air flow rate through the chamber, increased surface area of the skin due to the presence of residual hair stubble, and the higher temperature of the rat's skin surface.

Mathematical models with a large number of parameters, such as PBPK models, require sufficient sample collection intervals to adequately validate the model. It is especially critical that samples be collected during dynamic (changing) periods of the processes being studied. Thus, the present modeling effort could be substantially improved if additional samples were collected in the period between 0 and 30 min when both evaporation and absorption processes were ongoing.

For the purposes of human health risk assessment, the present PBPK model for the rat could be readily extended to the human in most respects. Physiological and physico-chemical parameters for the human are readily obtainable. However, one critical limitation exists. This limitation is the lack of appropriate metabolism parameters for either the rat or the primate regarding 1) the conversion of methanol to its toxic metabolite, formate, and 2) the conversion of formate to carbon dioxide. Unlike the rodent, formate accumulates in the primate (including the human) following methanol poisoning at high dose levels. It is this formate accumulation which accounts for the characteristic methanol toxicity in the human that is not observed, even at extraordinarily high methanol doses, in the rat (Tephly and McMartin, 1984). Determination of the metabolic parameters for formate accumulation would allow construction of a complete PBPK model useful for the assessment of human health risks due to methanol poisoning, regardless of route of exposure.

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Appendix A
Experimental Plan

Table A-1

Experimental Plan

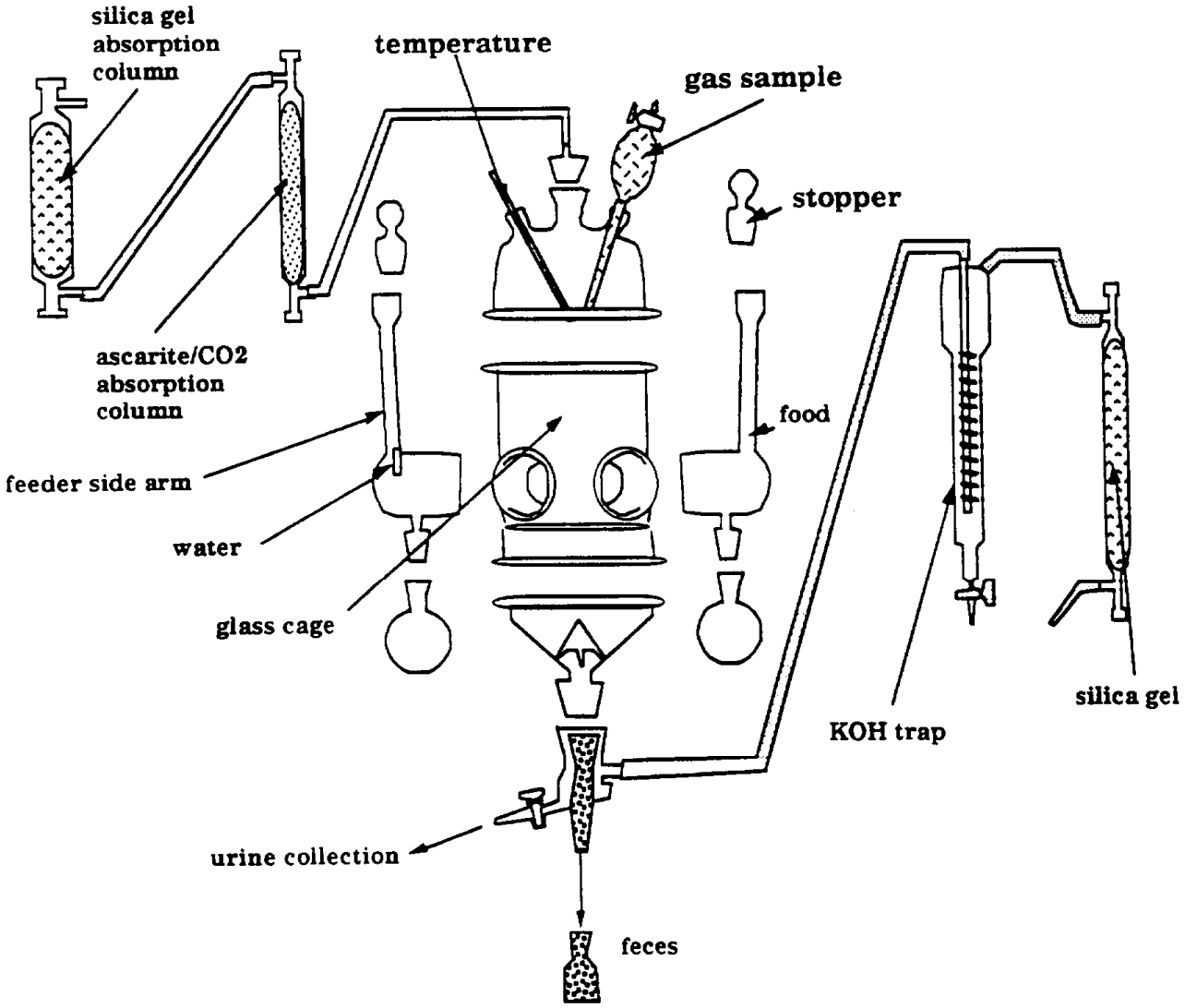
Exper #	Treatment Date	Experiment Duration (hours)	Route of Treatment	Rat I.D. ARB#	%Methanol In Mixture
1	6/19/1990	4-24	DERMAL	1-12	100
1	6/19/1990	24	DERMAL	13-16	100
2	6/25/1990	1	DERMAL	17-20	100
3	6/26/1990	4	DERMAL	21-24	100
4	6/27/1990	24	DERMAL	25-28	100
5	6/28/1990	24	SCU	29-32	100
6	7/05/1990	0.5	DERMAL	33-36	100
15	7/30/1990	48	DERMAL	69-72	100
7	7/09/1990	1	DERMAL	37-40	95
8	7/10/1990	4	DERMAL	41-44	95
9	7/11/1990	24	DERMAL	45-48	95
10	7/12/1990	24	SCU	49-52	95
11	7/23/1990	1	DERMAL	53-56	90
12	7/24/1990	4	DERMAL	57-60	90
13	7/25/1990	24	DERMAL	51-64	90
14	7/26/1990	24	SCU	65-68	90
16	8/06/1990	1	DERMAL	73-76	85
17	8/07/1990	4	DERMAL	77-80	85
18	8/08/1990	24	DERMAL	81-84	85
19	8/09/1990	24	SCU	85-88	85
20	8/13/1990	1	DERMAL	89-92	50
21	8/14/1990	4	DERMAL	93-96	50
22	8/15/1990	24	DERMAL	97-100	50
23	8/16/1990	24	SCU	101-104	50
24	8/30/1990	1	DERMAL	105-108	5
25	8/31/1990	4	DERMAL	109-112	5
26	9/04/1990	24	DERMAL	113-116	5
27	9/05/1990	24	SCU	117-120	5

Appendix B

Schematic of Metabolism Cage System

Figure B-1

Schematic of Metabolism Chamber System



Appendix C

Physiologically-Based Pharmacokinetic Model

A system of simultaneous, ordinary differential equations based on the mass balance of methanol and its end-metabolite, carbon dioxide, was written. These equations and corresponding assumptions are described in detail below. Specific values of the parameters used in the equations, along with references, are listed in Tables C-1 and C-2. All parameters were determined a priori from independent published literature sources, with the exception of the methanol evaporation rate constant (KEV) and the Michaelis constant for methanol metabolism (Km1). Thus only two parameters in the model were estimated. However, due to the small number of time points, these parameters could not be determined using computerized parameter estimation techniques. Instead, they were determined by manual fitting (visual inspection). The system of differential equations was coded in the simulation programming language, ACSL, and numerically integrated using the simulation software, SimuSolv (Dow Chemical Co., Midland, MI).

Dermal absorption: Numerous pharmacokinetic models of the dermal drug or toxicant absorption process have been developed by Guy et al. (1982, 1983, 1985), Albery and Hadgraft (1979), Guy and Hadgraft (1983), Stehle et al. (1989) and others. In general, these models are empirical compartmental models rather than physiologically-based models. The model of Guy et al. (1985) is representative. In this model four compartments are used, representing the skin surface, the stratum corneum, the viable epidermis, and the urine. Transfer rates between the compartments are assumed to follow first-order kinetics. The first-order rate constants are empirically-derived, that is, they cannot be derived a priori based on theoretical or physical/physiological considerations. Furthermore, because the rate constants in this, and similar models, are empirical, there is no basis for extrapolating the rate constant values obtained in such a model to other chemicals, other animal species, or even other concentrations of a given applied chemical. The utility of this model type in human health risk assessment is therefore limited. For this reason, a physiologically-based pharmacokinetic modeling approach was used in the present study.

The basis for most quantitative, kinetic analyses of the dermal absorption process is Fick's diffusion law. In simplest form this law may be expressed as:

Table C-1

Parameter Values Used in Physiologically-Based Pharmacokinetic Model

Parameter	Symbol	Value	Units	Reference
body weight	BW	320	g	Not applicable
liver volume	VLIV	0.0128	L	Arms and Travis (1988)
fat volume	VFAT	0.0224	L	Arms and Travis (1988)
volume of rapidly perfused tissue	VRPT	0.016	L	Arms and Travis (1988)
volume of slowly perfused tissue	VSPT	0.24	L	Arms and Travis (1988)
total blood volume	BV	0.016	L	Caster et al. (1956)
arterial blood volume	ABV	0.005	L	30% of total blood volume
venous blood volume	VBV	0.011	L	70% of total blood volume
cardiac output	QT	5.49	L/h	Popovic and Kent (1964)
liver blood flow	QL	1.37	L/h	Arms and Travis (1988)
fat blood flow	QF	0.5	L/h	Arms and Travis (1988)
rapidly perfused tissue blood flow	QR	2.8	L/h	Arms and Travis (1988)
slowly perfused tissue blood flow	QS	0.82	L/h	Arms and Travis (1988)
alveolar ventilation	QALV	4.61	L/h	84% of cardiac output (see text)
urine flow	QU	0.0007	L/h	Bivin et al. (1979)
urine/blood partition coeff.	PCU	1.3		Leaf and Zatman (1952)
bile flow	QBI	0.0008	L/h	Mehendale (1989)
bile/blood partition coeff.	PCBI	1		Yant and Schrenk (1937)
liver/blood partition coeff.	PCL	0.77		Yant and Schrenk (1937)
fat/blood partition coeff.	PCF	0.1		Yant and Schrenk (1937)
rap. per. tiss./blood partition coeff.	PCR	0.8		Yant and Schrenk (1937)
slow. per. tiss./blood partition coeff.	PCS	0.79		Yant and Schrenk (1937)
blood/air partition coeff.	PCB	1627		Fiserova-Bergerova and Diaz (1986)
Vmax	V1	0.3	mmol/h	Tephly et al. (1964)
Km	KM1	20	mM	Estimated parameter
dose of methanol applied to skin	M0	12	mmol	Not applicable
evaporation rate constant	KEV	7	per h	Estimated parameter
area of application site	A	25	cm2	Not applicable
conc. of pure methanol	CM	24.58	mmol/cm3	Not applicable
methanol diffusivity in strat. corneum	D	6.2E-05	cm2/h	Scheuplein and Blank (1973)
thickness of strat. corneum	THSC	1.8E-03	cm	Scheuplein and Blank (1973)
stratum corn:methanol partition coeff.	PC1	0.3		Scheuplein and Blank (1973)
stratum corn:blood partition coeff.	PC2	0.3		Scheuplein and Blank (1973)
metabolism chamber volume	CHV	8.6	L	Not applicable
airflow through chamber	QCH	30	L/h	Not applicable

Table C-2

Notation Index for PBPK Model

Symbol	Parameter	Value	Units	Reference
A	area of application site	25	cm2	Not applicable
ABV	arterial blood volume	0.005	L	30% of total blood volume
BV	total blood volume	0.016	L	Caster et al. (1956)
BW	body weight	320	g	Not applicable
CHV	metabolism chamber volume	8.6	L	Not applicable
CM	conc. of pure methanol	24.58	mmol/cm3	Not applicable
D	methanol diffusivity in strat. corneum	6.2E-05	cm2/hr	Scheuplein and Blank (1973)
KEV	evaporation rate constant	7	per hr	Estimated parameter
KM1	Km	20	mM	Estimated parameter
MO	dose of methanol applied to skin	12	mmol	Not applicable
PC1	stratum corn:methanol partition coeff.	0.3		Scheuplein and Blank (1973)
PC2	stratum corn:blood partition coeff.	0.3		Scheuplein and Blank (1973)
PCB	blood/air partition coeff.	1627		Fiserova-Bergerova and Diaz (1986)
PCBI	bile/blood partition coeff.	1		Yant and Schrenk (1937)
PCL	liver/blood partition coeff.	0.77		Yant and Schrenk (1937)
PCF	fat/blood partition coeff.	0.1		Yant and Schrenk (1937)
PCR	rap. per. tiss./blood partition coeff.	0.8		Yant and Schrenk (1937)
PCS	slow. per. tiss./blood partition coeff.	0.79		Yant and Schrenk (1937)
PCU	urine/blood partition coeff.	1.3		Leaf and Zatman (1952)
QALV	alveolar ventilation	4.61	L/hr	84% of cardiac output (see text)
QB	bile flow	0.0008	L/hr	Mehendale (1989)
QCH	airflow through chamber	30	L/hr	Not applicable
QF	fat blood flow	0.5	L/hr	Arms and Travis (1988)
QL	liver blood flow	1.37	L/hr	Arms and Travis (1988)
QR	rapidly perfused tissue blood flow	2.8	L/hr	Arms and Travis (1988)
QS	slowly perfused tissue blood flow	0.82	L/hr	Arms and Travis (1988)
QU	urine flow	0.0007	L/hr	Bivin et al. (1979)
THSC	thickness of strat. corneum	1.8E-03	cm	Scheuplein and Blank (1973)
V1	Vmax	0.3	mmol/hr	Tephly et al. (1964)
VBV	venous blood volume	0.011	L	70% of total blood volume
VFAT	fat volume	0.0224	L	Arms and Travis (1988)
VLIV	liver volume	0.0128	L	Arms and Travis (1988)
VRPT	volume of rapidly perfused tissue	0.016	L	Arms and Travis (1988)
VSPT	volume of slowly perfused tissue	0.24	L	Arms and Travis (1988)

$$J = \frac{D \times A \times (C_1 - C_2)}{THSC} \qquad (2)$$

where J is the dermal absorption rate (mmol/h) of chemical applied to the skin surface, A is the area of skin treated (cm^2), D is the diffusion coefficient of the chemical in the stratum corneum (cm^2/h), C_1 is the concentration of chemical at the skin surface side of the stratum corneum ($mmol/cm^3$), C_2 is the concentration of the chemical on the blood side of the stratum corneum ($mmol/cm^3$), and THSC is the thickness of the stratum corneum (cm). This equation states that the rate of absorption is proportional to the concentration gradient of the chemical across the stratum corneum and the diffusion coefficient of the chemical in the stratum corneum.

In typical applications it is usually assumed that the stratum corneum layer of the skin is the primary resistance layer to absorption. This appears to be a realistic assumption for most chemicals (Scheuplein and Blank, 1971). For example, the diffusion coefficient of the alcohols and water in the stratum corneum is four orders of magnitude lower than it is for the viable dermis. Another important assumption of Equation 2 is that the resistance layer (the stratum corneum) is inert with respect to the applied chemical, that is, the stratum corneum has no special affinity for the applied chemical. Scheuplein and Blank (1971) have pointed out that this is not the case for most chemicals. Rather, the effective concentration gradient is established based on the interfacial concentration of the applied chemical in the stratum corneum rather than on the concentration in the applied solution per se. These investigators have therefore modified Equation 2 by adding the stratum corneum:solution partition coefficient, here notated PC1, to reflect the differential affinity of the stratum corneum for the applied chemical. Thus, PC1 is equal to:

$$PC1 = \frac{CSC}{CS} \qquad (3)$$

where CSC is the concentration of the applied chemical in the stratum corneum at equilibrium and CS is the concentration of the applied chemical in the applied solution at equilibrium. Inserting Equation 3 into Equation 4:

$$J = \frac{A \times PC1 \times D \times (CS_1 - CS_2)}{THSC} \qquad (4)$$

Here CS_1 is equal to the concentration of chemical in solution on the upgradient side of the stratum corneum and CS_2 is the concentration of chemical in solution on the downgradient side of the stratum corneum. Thus, Equation 4 converts the concentration difference from that between the two compartments on either side of the stratum corneum to that between the interfacial layers of the stratum corneum. In Equation 4, the term "(PC1 × D/THSC)" is usually replaced by the permeability coefficient or constant, K_p (cm/h).

A theoretical weakness of Equation 4 is that it assumes that the partition coefficient for the chemical between the vehicle and the stratum corneum is the same as the partition coefficient for the chemical between the stratum corneum and the "sink" tissue (blood or other tissue on the "downstream" side of the stratum corneum). Riggs (1963) has taken this into account explicitly though the use of an additional partition coefficient, here notated as PC2, which represents the partitioning of the applied chemical between the stratum corneum and the "downstream" tissue, for example, the blood:

$$PC2 = \frac{CSC}{CTISS} \qquad (5)$$

where CTISS is the equilibrium concentration of the applied chemical in the tissue immediately downgradient of the stratum corneum and CSC is defined as above. Thus, Riggs derived the following expression:

$$J = \frac{D \times A}{THSC} \times (PC1 \times C_1 - PC2 \times C_2) \qquad (6)$$

This form of Fick's law of diffusion was incorporated in the physiologically-based pharmacokinetic model developed in this study and is described in more detail below.

In the PBPK model, therefore, the absorption of methanol from the skin was assumed to be proportional to the methanol concentration difference between the dermal application site (CM, mM) and the mixed venous blood (CVEN, mM). More exactly, this concentration difference is represented by the concentration of methanol in the outer layer of stratum corneum on the dermal side and the inner layer of stratum corneum on the venous blood side. These concentrations are in turn dependent on the partition coefficient between pure methanol and stratum corneum (PC1) on the dermal side and the partition coefficient for methanol partitioning between stratum corneum and venous blood (PC2). The stratum corneum of the skin was considered to be the primary resistance layer to absorption, as demonstrated by Scheuplein and Blank (1973). The dermal absorption of methanol is also a function of the diffusivity (diffusion coefficient) of methanol in the stratum corneum (D, cm^2/s), the surface area of the stratum corneum treated (A, cm^2), and the thickness of the stratum corneum (THSC, cm). The rate of absorption (DABSDT, mmol/h) as given by (Riggs, 1970) and used in this PB-PK model, is therefore:

$$DABSDT = \left(\frac{D \times A}{THSC}\right) \times (PC1 \times CM - PC2 \times CVEN) \qquad (7)$$

The concentration CM was assumed to be constant thoughout the absorption process. The value of PC1, of 0.3, was obtained directly from Scheuplein and Blank (1973), however, the value of PC2 (the stratum corneum:blood partition coefficient) was derived by assuming first, that the partition coefficient between pure methanol and blood is 1 and then multiplying this value by PC1 (the stratum corneum:pure methanol partition coefficient). Thus, both PC1 and PC2 are

equal to 0.3. Integration of Equation 7 results in the cumulative amount of methanol absorbed at any given time.

Evaporation: The instantaneous rate of evaporation of methanol (DEVAP, mmol/h) from the dermal application site was assumed to be a first-order process:

$$DEVAP = KEV \times AM \qquad (8)$$

where KEV (per h) is the first-order evaporation rate constant and AM is the amount of methanol at the site (mmol). A simple experiment was conducted to verify the first-order nature of this process. The weight loss from an aliquot of pure methanol placed on a glass plate was measured over time. The data were linear when plotted on semilog paper indicating a first order process. The value of KEV was determined by data fitting. Integration of Equation 8 results in the cumulative amount of methanol evaporated at any given time (AEV, mmol).

Simultaneous evaporation and absorption: The rate of methanol loss from the application site due to simultaneous absorption and evaporation (DAMDT, mmol/h) was described as follows:

$$DAMDT = -(DEVAP+DABSDT) \qquad (9)$$

Integration of Equation 9 results in the total loss of methanol from the dermal application site at any given time (AM, mmol).

Exhalation of methanol: The instantaneous rate of methanol loss via exhalation (DAEXDT, mmol/h) was modeled as:

$$DAEXDT = QALV \times CALV \qquad (10)$$

where QALV is the rate of alveolar ventilation (L/h), CALV (the alveolar gas concentration of methanol) equals the arterial blood concentration, CA (mM), divided by the blood:gas partition coefficient, PCB. QALV was determined using the experimentally determined cardiac output estimated by Popovic and Kent (1964) and by assuming a ventilation:perfusion ratio of 0.84 (Staub, 1991). Integration of Equation 10 results in the cumulative amount of methanol exhaled at any given time.

Venous blood: The volume of the venous blood compartment was assumed to constitute 70% of the total blood volume (Guyton, 1981). Methanol absorbed though the skin was assumed to enter the venous blood and mix with the venous blood flows from all other tissue groups. Methanol is lost from the venous blood via the mixed venous outflow (QT × CVEN), which enters the arterial blood compartment. Thus, the change in the methanol content of the venous blood (DVBDT, mmol/h) was expressed as:

$$DVBDT = DABSDT+QF\times CVFAT+QR\times CVRPT+QS\times CVSPT+QL\times CVLIV-QT\times CVEN \quad (11)$$

where QF, QR, QS, and QL (L/h) are the venous blood flows from the fat, rapidly perfused tissue, slowly perfused tissue and liver, respectively and CVFAT, CVRPT, CVSPT, CVLIV, are the representive methanol concentrations in the venous blood leaving the tissue. QT is the total cardiac output. Integration of Equation 11 results in the amount of methanol in the venous blood compartment at any given time.

Urinary and biliary excretion: The instantaneous urinary excretion rate (DUDT, mmol/h) and biliary excretion rate (DBDT, mmol/h) were modeled as continuous physiological flows:

$$DUDT = QU\times CU \quad (12)$$

$$DBDT = QBI \times CB \qquad (13)$$

where QU and QB are the flows of urine and bile (L/h), CU is the urine concentration of methanol (mM), and CB is the bile concentration of methanol (mM). CU in turn is equal to CA times the urine:blood partition coefficient, PCU. Similarly, CB equals CA times the bile:blood partition coefficient, PBI. Integration of Equations 12 and 13 result in the cumulative amounts of methanol eliminated via urine and bile, respectively, at any given time.

Arterial blood: The arterial blood volume was assumed to constitute 30% of the total blood volume (Guyton, 1981). The methanol mass balance on the arterial blood pool includes input from the mixed venous flow (QT × CVEN), loss via arterial outflow to the tissues (QT × CA), loss via urinary excretion (DUDT), and loss via exhalation (QALV × CALV):

$$DABDT = QT \times CVEN - QT \times CA - DUDT - QALV \times CALV \qquad (14)$$

Integration of Equation 14 results in the amount of methanol in the arterial blood pool at any given time.

Nonmetabolizing tissues: The representative model equation used to describe the rate of methanol uptake by fat, RPT or SPT is:

$$DTISDT = QTIS \times (CA - CTVIS) \qquad (15)$$

where QTIS is the blood flow rate to the tissue (or QF, QR, QS) CA is the arterial concentration of methanol and CVTIS is the venous blood methanol concentration (or CVFAT, CVRTP, CVSPT).

The venous blood methanol concentration was assumed to be in equilibrium with the tissue methanol concentration as described by the tissue:blood partition coefficient. Hence CVTIS was replaced with CTIS/PCTIS, where CTIS is the tissue concentration of methanol and PCTIS is the blood:tissue partition coefficient for methanol. Integration of Equation 15 results in the amount of methanol in the tissue at any given time. The tissue concentration was then determined by dividing the amount of methanol in the tissue by the volume of the tissue.

Liver: The elimination of methanol from the liver was assumed to occur via metabolism (DADT, mmol/h) and biliary excretion (DBDT). The metabolism of methanol is complex (see Figure 2), consisting of several short-lived intermediates and the end- metabolite carbon dioxide. In the monkey, the methanol metabolic intermediate, formate, accumulates in the blood following high doses of methanol (Tephly and McMartin, 1984). This indicates that formate is eliminated by a saturable process in this species. In the rat, however, even extraordinarily high doses of methanol (6 g/kg) result in no significant accumulation of formate, even though methanol appears to be converted to formate at the same rate as in the monkey (Tephly and McMartin, 1984). This suggests that methanol conversion to formate rather than formate elimination is rate-limiting in the rat. The kinetics of methanol elimination have been shown to follow nonlinear (Michaelis-Menten) kinetics (Tephly and McMartin, 1984). If it is assumed that methanol conversion to formate is rate-limiting (i.e., all subsequent conversions are much faster than this initial biotransformation), than the metabolism of methanol to the end-metabolite carbon dioxide may be simplified to a single Michaelis-Menten term:

$$DADT = \frac{V1 \times CVLIV}{Km1 + CVLIV} \qquad (16)$$

where DADT is the rate of of methanol conversion to carbon dioxide in the liver, CVLIV is the liver concentration of methanol, V1 is the maximum rate of methanol metabolism to carbon dioxide for the entire liver (mmol/h), and Km1 is the methanol concentration at which the rate of metabolism is half-maximal (mM). Integration of Equation 16 results in the amount of carbon

dioxide produced at any given time. The parameters, V1 and Km1, in this case are assumed to represent the overall kinetics for the conversion of methanol though several short-lived intermediates to carbon dioxide.

The mass balance on the liver incorporates uptake from the arterial blood, loss via metabolism and loss via biliary excretion:

$$DLIVDT = QL \times (CA - CVLIV) - DADT - DBDT \qquad (17)$$

Where DLIVDT is the rate of change of methanol amount in the liver (mmol/h), DADT is the rate of liver metabolism of methanol, and DBDT is the biliary excretion rate. Integration of Equation 17 results in the amount of methanol in the liver at any given time.

Metabolism Chamber: The rate of change of the amount of methanol in the metabolism chamber air (DACHDT, mmol) is:

$$DACHDT = KEV \times AM + QALV \times CALV - QCH \times CCH \qquad (18)$$

where QCH is the airflow rate though the chamber (L/h), and CCH is the methanol concentration in the chamber air (mmol/L). Integration of Equation 18 results in the amount of methanol in the chamber at any given time. Division of the amount of methanol in the chamber by the volume of the chamber results in the methanol concentration in the chamber (CCH, mM).

Methanol exiting metabolism chamber: The rate of methanol loss from the metabolism chamber (AEXCH, mmol/h) is expressed as:

$$AEXCH = QCH \times CCH \qquad (19)$$

Integration of Equation 19 results in the cumulative amount of methanol lost from the chamber at any given time.

Appendix D

Estimation of Exhaled Methanol/Carbon Dioxide Ratio

The exhaled methanol could not be measured directly because the methanol collected in the methanol trap (water bubblers) is a mixture of the methanol evaporated directly from the skin as well as the methanol exhaled by the animal after absorption into the systemic circulation. Therefore, an additional treatment was conducted in which rats were injected with ^{14}C-methanol subcutaneously (scu). The fraction of injected methanol exhaled as determined from the scu treatment was assumed to be same for the dermal routes of exposure. The experimental design of the scu treatment was as follows:

1) Groups of four rats were injected subcutaneously with either pure methanol or one of the methanol/gasoline mixtures at doses equal to 10% of the dermally applied dose.

2) Following injection, the rats were placed in the glass metabolism cages and the exhaled methanol and CO_2 collected as described for the dermally-treated rats.

3) The ratio of µg methanol collected to µg $^{14}CO_2$ collected was calculated for each rat at each time period.

4) The mean methanol/CO_2 ratio was multiplied by the µg of $^{14}CO_2$ collected during the dermal study in order to estimate the µg of methanol exhaled.

Table D-1

Ratio of Methanol/Carbon Dioxide Exhaled in Rats Treated With Methanol/Gasoline Mixtures Subcutaneously

Methanol/Gasoline Mixture (%/%)	Ratio of Methanol/Carbon Dioxide Exhaled		
	Time Post-Application (h)		
	1	4	24
100	0.5	0.18	0.09
95/5	0.41	0.19	0.15
90/10	0.72	0.20	0.16
85/15	0.62	0.20	0.11
50/50	0.44	0.13	0.10
5/95	0.18	0.07	0.07

Appendix E

Mass Balance Calculation Formula

Figure E-1

Mass Balance Calculation for Determining Amount of Applied

Methanol in Carcass (Primarily Muscle)

$$CAR = TDABS - TISS - CO2 - METH - URINE - FECES$$

Where:

CAR = % of absorbed dose in carcass (primarily muscle tissue)

TABS = % of applied methanol dermally absorbed

TISS = % of absorbed methanol found in selected tissues

CO2 = % of absorbed methanol exhaled as CO_2

METH = % of absorbed methanol exhaled as methanol

URINE = % of absorbed methanol excreted in urine

FECES = % of absorbed methanol excreted in feces

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O.G. Raabe, M.A. Al-Bayati, P.D. Shulec, F. Gielow, D. Uyeminami, and N. Shimasaki

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Institute of Toxicology and Environmental Health
School of Veterinary Medicine
University of California
Davis, CA 95616

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13. ABSTRACT *(Maximum 200 Words)*

The objective of this study was to determine the effect of dermally applied methanol/gasoline mixtures on ^{14}C-methanol metabolism in Sprague-Dawley rats. Following applications of 100% methanol or methanol/gasoline mixtures (95, 90, 85, 50 or 5% methanol), methanol vapor, carbon dioxide, and excreta were collected, and the animals sacrificed to quantify levels of ^{14}C in blood and major tissues. Mean dermal absorption efficiencies of methanol were 24.8, 31.6, 24.5, 27.0, 35.7 and 58.1% for the 100, 95, 90, 85, 50 and 5% methanol mixtures, respectively. Only the absorption efficiencies of the 5% and 50% methanol mixtures were statistically significant relative to 100% methanol. At high gasoline concentrations, increased levels of nonpolar compounds may act to force methanol into the stratum corneum of the skin. Alternatively, gasoline may damage the skin and enhance methanol absorption, or reduce rates of methanol evaporation. For all fuel mixtures, 68% of the dermally- applied methanol evaporated within 60 minutes. Exhalation of carbon dioxide was the main route of ^{14}C-methanol elimination (about 50%); about 5% was exhaled as methanol, 4 to 11% was eliminated as uncharacterized ^{14}C in excreta, and 30-40% remained in the animal. With the possible exception of the kidney, tissue ^{14}C levels were not elevated relative to plasma concentrations after 24 hr. A pharmacokinetic model was used to simulate the main aspects of methanol absorption and disposition.

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