Risk Analysis, Vol. 17, No. 6, 1997

Route-to-Route Extrapolation of the Toxic Potency of MTBE

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Received April 9, 1996; revised December 6, 1996

MTBE is a volatile organic compound used as an oxygenating agent in gasoline. Inhalation from fumes while refueling automobiles is the principle route of exposure for humans, and toxicity by this route has been well studied. Oral exposures to MTBE exist as well, primarily due to groundwater contamination from leaking stationary sources, such as underground storage tanks. Assessing the potential public health impacts of oral exposures to MTBE is problematic because drinking water studies do not exist for MTBE, and the few oil-gavage studies from which a risk assessment could be derived are limited. This paper evaluates the suitability of the MTBE database for conducting an inhalation route-to-oral route extrapolation of toxicity. This includes evaluating the similarity of critical effect between these two routes, quantifiable differences in absorption, distribution, metabolism, and excretion, and sufficiency of toxicity data by the inhalation route. We conclude that such an extrapolation is appropriate and have validated the extrapolation by finding comparable toxicity between a subchronic gavage oral bioassay and oral doses we extrapolate from a subchronic inhalation bioassay. Our results are extended to the 2-year inhalation toxicity study by Chun *et al.* (1992) in which rats were exposed to 0, 400, 3000, or 8000 ppm MTBE for 6 hr/d, 5 d/wk. We have estimated the equivalent oral doses to be 0, 130, 940, or 2700 mg/kg/d. These equivalent doses may be useful in conducting noncancer and cancer risk assessments.

KEY WORDS: MTBE; route-to-route; extrapolation; groundwater.

1. INTRODUCTION

Ideally, toxicity studies in experimental animals by one route of exposure should be used to estimate the likely health risk to humans by the same route of exposure. This avoids possible complications in the assessment of risk from, for example, inter-route differences in deposition/absorption of the chemical, its distribution, metabolism and excretion, and its possible portal of entry effects. Where studies by one route of exposure are not available, however, information may be gleaned from an analysis of studies by other routes of exposure. In some cases, justification may exist for using such data in a route-to-route extrapolation.

Such extrapolation was proposed for example, by Stokinger and Woodward[1] in the estimation of water criteria when sufficient oral toxicity data were not available by way of a Threshold Limit Value using the following equation:

$$\text{Water Criterion (mg/l)} = \text{TLV (mg/m}^3\text{)} \times 10\text{m}^3\text{/day} \times \text{(Ratio of Inhaled to Ingested Absorption Rates)} \div 2 \text{ liters of water ingested/day} \quad (1)$$

However, limitations of this method and others based on this approach have been noted. For example, U.S. Environmental Protection Agency (EPA)[2] and Pepelko and Withey[3] extensively discussed the limitations of the Stokinger and Woodward method stating that measured absorption factors are generally lacking, the TLV may not be based on systemic toxicity, extensive hepatic metabolism may reduce the systemic toxicity by the oral route (i.e., first pass effect), temporal relationships of blood levels after administration are not consid-

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0272-4332/97/1200-0717$12.50/1

ered, and the deposition of particulate material in the lung available for absorption is dependent on particle size.

The EPA has developed tentative positions for route-to-route extrapolation for both cancer and noncancer toxicity.[4] Specifically, the potential for toxicity manifested by one route is considered to be relevant to considerations of other routes of exposure, unless convincing evidence exists to the contrary. Evidence to consider include potential differences in absorption or metabolism and whenever appropriate data are available, the quantitative impacts of these differences on potential toxicities should be delineated. Route-to-route extrapolation was also the focus of a comprehensive workshop.[5] This workshop covered the topics of structure and function of barriers, parameters associated with absorption of toxicants, critical factors such as metabolic fate of inhaled chemicals and case studies.

As presented in depth in other papers in this issue, the toxicity database for MTBE after oral exposure is not sufficient to develop a dose–response assessment for either cancer or noncancer toxicity, whereas the database for MTBE by the inhalation route of exposure is extensive.[6,7] The focus that has been placed on the toxicity of MTBE by inhalation reflects this being the primary route of exposure to humans (i.e., through inhalation of fumes from gasoline containing MTBE). However, the potential also exists for oral exposure of humans to MTBE. In particular, MTBE has been detected in groundwater from leaking underground storage tanks, and the potential for contamination of drinking water is of concern. The purpose of this paper, then, is to determine whether the extrapolation of toxicity data from the inhalation route of exposure to the oral route is feasible for MTBE and how such an extrapolation should be done.

2. CRITERIA TO DETERMINE WHETHER ROUTE-TO-ROUTE EXTRAPOLATION IS APPROPRIATE

One approach to route-to-route extrapolation is to use physiologically-based pharmacokinetic (PBPK) models. A PBPK model for MTBE and TBA in rats has been published recently.[8] The model was able to predict gas uptake data and levels of MTBE in the blood following exposure of rats by inhalation, iv, and oral routes. The pharmacokinetic handling of TBA in the rat was found to be more complex than that of MTBE, however, and the authors concluded that further studies are needed on TBA kinetics in order to better refine the model.

In order to use a PBPK model for route-to-route extrapolation of the toxicological or carcinogenic effects of MTBE, a number of issues must be decided upon; for example, whether the extrapolation should be based upon levels of TBA or MTBE, and if extrapolation is based on MTBE, whether the area-under-the-curve for blood levels of MTBE should be used versus total amount of MTBE metabolized by the liver. It is also noted that the oral data upon which the published model is based are from gavage administration of MTBE, and that the rate of oral absorption following gavage administration may be quite different from that following exposure in drinking water. That may also have a bearing on the application of the PBPK model for extrapolation from an inhalation exposure to a drinking water scenario. These issues are currently being investigated along with further refinement of the PBPK model.[2]

The PBPK model for MTBE[8] can also be used for cross-species extrapolation. MTBE-specific parameters (e.g., for partition coefficients and metabolic rate constants) are not currently available for humans, but the model for rats has been used in combination with allometric scaling for humans. Borghoff *et al.*[8] have noted that this adapted model accurately predicts MTBE blood levels in humans during exposure, but underestimates blood levels upon termination of exposure, further emphasizing the need for specific chemical parameters for humans. The refinement of the PBPK model for MTBE in rats and humans, then, will allow for interspecies extrapolation by integrating internal doses with observed biological responses, and in intraspecies extrapolation between various routes of exposure. Application of such a model will ultimately result in increased confidence in the extrapolation.

In the absence of a PBPK model, criteria have been developed for evaluating the appropriateness of route-to-route extrapolation for specific chemicals. For example, Pepelko,[9] based in part on Pepelko and Withey,[3] suggests the following seven points to consider when performing such an extrapolation: (1) absorption efficiency is the same between routes or the difference is known and can be quantified; (2) half-life of the chemical is long; (3) first pass effects are minimal; (4) no significant chemical transformation by intestinal microflora or pulmonary macrophages; (5) critical target tissue is not at the portal of entry; (6) chemical is relatively soluble in body fluids; and (7) adequate chronic toxicity data are available for the route used as a basis for extrapolation.

In the development of methods for the determination of a Reference Concentration (RfC), EPA[10] speci-

[2] Borghoff, personal communication.

fied criteria for route-to-route extrapolations, which can be attempted if portal of entry effects in the lung can be ruled out.[3] In such cases EPA indicates that estimates of equivalent doses can be based upon the following criteria: (1) available toxicokinetic data for the routes of interest; (2) measurements of absorption efficiency by each route of interest; (3) comparative excretion data when the associated metabolic pathways are equivalent by each route of interest; and (4) comparative systemic toxicity data when such data indicate equivalent effects by each route of interest.

The concentration of parent chemical or its active metabolite at the site of critical effect is the most important piece of knowledge in any route-to-route extrapolation within a given specie. Thus, comparative examination of absorption, distribution, metabolism and excretion between the inhalation and oral routes of a given specie are evaluated in this paper to determine whether it is appropriate to conduct a route-to-route extrapolation for MTBE.

3. ANALYSIS OF TOXICOKINETIC DATA FOR MTBE

3.1. Absorption

Studies of humans exposed to MTBE indicates that significant absorption occurs from the lungs. CDC[11] investigated increases in blood levels of MTBE in occupationally exposed workers (e.g., mechanics and gas station attendants) in Fairbanks, Alaska. Air levels of MTBE were found to be strongly correlated ($r = 0.9$; $p = 0.0001$) with changes in blood concentrations over the period of exposure.

Johanson *et al.*[12] exposed healthy male volunteers to MTBE vapors for 2 hr during light physical work at concentrations of 5, 25, or 50 ppm. The authors estimated that 32–41% of the inhaled MTBE was absorbed. Cain *et al.*[13] exposed four volunteers to 1.7 ppm MTBE for 1 hr.[13] Blood levels were found to increase from 0.83 μg/l (preexposure) to 17.1 μg/l at the end of the hour. One hour post-exposure, blood levels of MTBE had decreased to 6.32 μg/l. Blood levels of TBA were also monitored, but were found to be highly variable, perhaps due to analytical difficulties. Prah *et al.*[14] exposed two volunteers (one male and one female) to 1.39 ppm (5.0 mg/m^3) MTBE for 1 hr, a concentration chosen to approximate that experienced during the refueling of an automobile. A rapid rise in blood MTBE was observed, with peak levels of 8.2 ppb (male) and 14.7 ppb (female) being achieved at the point of cessation of exposure. Blood MTBE subsequently declined rapidly with an elimination half-time of about 36 min. Sampling continued for a total of 7 hr, at which point the blood MTBE levels had fallen to 0.2 ppb (male) and 0.6 ppb (female). While the MTBE levels were falling, blood TBA concentrations continued to gradually increase until a plateau of 7–10 ppb was achieved.

In studies of laboratory animals, MTBE has been found to be rapidly and extensively absorbed following both oral and inhalation exposures. Bio-Research Labs[15] determined the AUC for MTBE in rats following both i.v. and gavage (in 0.9% saline) administration. This information is presented in Table I. It was found that the AUC ($t = 0 \rightarrow$) following i.v. administration was actually lower than the AUC following gavage administration of the same dose. The authors surmised that this was due to the more rapid exhalation of MTBE following i.v. administration, which is determined in part by the concentration of MTBE in blood and the blood:air partition coefficient for MTBE. The fact that the AUC following oral administration is in fact higher than the corresponding AUC for i.v. administration suggests that the MTBE was completely absorbed from the GI tract.

Bio-Research Labs[16] measured the recovery of radioactivity in urine, feces, expired air, and the carcasses of rats exposed to i.v. doses (single or repeated) of 40 mg/kg, or gavage doses (in 0.9% saline) of 40 or 400 mg/kg ^{14}C-MTBE. Collection of samples continued for 48 hr post-exposure. In both i.v. and oral groups, fecal excretion accounted for ≤1% of the administered dose, indicating virtually complete uptake from the GI tract. Maximal plasma concentrations of MTBE were achieved within 15 min of administration of oral doses of 40 or 400 mg/kg MTBE.

Exposure of rats to MTBE by nose-only inhalation for 6 hr resulted in maximal plasma concentrations of MTBE at 4–6 hr, and TBA at 6–6.5 hr, during exposure.[17] The maximal concentration f MTBE following exposure to 400 ppm was ~15 μg/ml, and following exposure to 8000 ppm was ~560 μg/ml. TBA levels in the plasma increased more gradually, with a maximal concentration of ~39 μg/ml following exposure to 400 ppm, and a maximal concentration of 536 μg/ml (males) or 245 μg/ml (females) following exposure to 8000 ppm.

[3] Several investigators have mentioned this criterion before. However, it was not until EPA estimated a number of RfCs that it realized that the respiratory system was the area of the critical effect for about 1/2 of all RfCs on its Integrated Risk Information System (IRIS) (Dourson, unpublished data). This realization made the prospect of route-to-route extrapolation more dependent on the nature of the critical effect after different routes of exposure.

Table I. Comparison of AUCs for MTBE and TBA Following Different Exposure Regimens

Study	Route	Dose	Sex	MTBE AUC (μg.hr/ml) $t = 0\rightarrow$	MTBE AUC (μg.hr/ml) $t = 6\rightarrow$	TBA AUC (μg.hr/ml) $t = 0\rightarrow$	TBA AUC (μg.hr/ml) $t = 6\rightarrow$
Bio-Research[24]	iv	40 mg/kg	M	10.7		26.7	
			F	7.9		32.2	
	oral	40 mg/kg	M	17.0		39.0	
			F	12.5		36.7	
		400 mg/kg	M	230		304	
			F	193		289	
Bio-Research[38]	inhal.single	400 ppm	M	84.3	10.5	404	116
			F	77.9	11.8	374	94.2
		8000 ppm	M	2960	406	6010	1790
			F	2870	369	2550	574
	inhale.repeat	400 ppm	M	—	6.7	—	127
			F	—	6.3	—	125

During a prolonged exposure to lower concentrations, MTBE levels in blood continue to rise for many weeks. Savolainen *et al.*[18] exposed rats to 50, 100, or 300 ppm MTBE for 6 hr/d, 5 d/wk for 2–15 wk. Peak blood concentrations were reached at 6 wk for the 50 and 100 ppm groups (0.97 and 2.1 μg/ml, respectively), but the peak blood concentration for the 300 ppm group was not reached until 15 wk (15 μg/ml).

3.2. Distribution and Retention

No studies have been performed that have specifically examined the distribution of MTBE following oral exposure. Kinetic data on absorption and excretion, however, have shown that MTBE is widely distributed and does not accumulate to a significant degree in any tissue.

Only one study is available which measured tissue retention of MTBE following inhalation exposure. Bio-Research Labs[19] exposed Fischer 344 rats to ^{14}C-MTBE at concentrations of 400 or 8000 ppm for 6 hr or 400 ppm for 6 hr/d for 15 d (the first 14 of which were to unlabeled MTBE). The rats were sacrificed 48 hr post-exposure, and radioactivity was measured in blood, liver, kidney, lungs, heart, brain, gonads, bone, fat, muscle, and skin. In both the single and 15-d exposures, mean radioactivity in tissues was very low (<1% of total) except for skin, which was likely due to contamination.

In Sprague-Dawley rats that received an i.p. dose of 232 mg/kg ^{14}C-MTBE, the total accumulation of radioactivity in tissues averaged 3.39%, 1.94%, and 1.14% of the administered dose after 15 min, 6 hr, and 24 hr, respectively.[20] In F344 rats administered a single i.v. dose of 40 mg/kg ^{14}C-MTBE, less than 2% of the radioactive dose was found in tissues and the carcass after 48 hr, and less than 1% was found after 7 d.[19] These studies each demonstrate a lack of accumulation of MTBE in tissues following acute exposures.

3.3. Metabolism

The oxidative metabolism of MTBE by cytochrome P450s results in the generation of *t*-butanol (TBA) and formaldehyde.[21] TBA is further metabolized to 2-methyl-1,2-propanediol and alpha-hydroxyisobutyric acid. The metabolism of MTBE does not appear to be route-dependent. Bio-Research Labs[15–17,19] investigated the metabolism of ^{14}C-MTBE in F344 rats following inhalation, oral, dermal, and intravenous routes of exposure. For all routes of exposure, most of the recovered radioactivity was in expired air, primarily as unchanged MTBE and *t*-butanol. Four urinary metabolites were found, but only two were identified: 2-methyl-1,2-propanediol and alpha-hydroxyisobutyric acid. As sampling time was increased, the proportion of alpha-hydroxyisobutyric acid increased compared with 2-methyl-1,2-propanediol, suggesting that the diol is an intermediate in the formation of the acid.

Saturation of the metabolism of MTBE to TBA has been demonstrated following both oral and inhalation exposures.[15,17] As presented in Table I, a tenfold increase in oral dose was associated with a greater-than-tenfold increase in the AUC for MTBE, and a less-than-tenfold increase in the AUC for TBA. Similarly, for a 20-fold increase in inhalation concentration, there was a greater-than-20-fold increase in the AUC for MTBE, and a less-than-20-fold increase in the AUC for TBA. In each of these cases, this difference is believed

to be due to saturation of the enzymes that catalyze the metabolism of MTBE to TBA.

Table I also presents data from single 6-hr inhalation exposures of rats to 400 ppm MTBE and repeat exposures (6 hr/d for 15 d) to the same concentration. Following repeated exposure, the AUC for MTBE in blood was found to be somewhat lower and the AUC for TBA somewhat higher than the corresponding values following a single inhalation exposure. This suggests that exposure to MTBE results in induction of its own metabolism.

3.4. Excretion

Following exposure by either the oral or inhalation route, the excretion of MTBE is rapid and virtually complete, with elimination occurring mainly through the lungs and the kidneys. Johanson *et al.*[12] exposed healthy male volunteers to MTBE vapors for 2 hr at concentrations of 5, 25, or 50 ppm. High post-exposure exhalation (18–34% of the MTBE taken up) was reported. Less than 1% of uptake was recovered as TBA in urine.

In a study by Bio-Research Labs,[16] rats were administered ^{14}C-MTBE by the intravenous route (40 mg/kg as a single dose or as a daily dose for 15 days) or by the oral route (single gavage doses of 40 or 400 mg/kg in 0.9% saline). The major routes of excretion were expired air and urine. As the dose was increased, there was a shift in excretion with less being excreted in the urine and more being excreted in expired air. This shift has been attributed to saturation of metabolic pathways. Excretion was rapid in this study, with virtually complete elimination in expired air occurring within 6 hr, and in urine within 36 hr.

Expired air was collected and analyzed during the first 6 hr post-exposure in the study by Bio-Research Labs.[16] The majority of exhaled radioactivity was collected within the first 3 hr. The unchanged parent compound accounted for 94–98% of the expired radioactivity. In the low-dose oral group, the fraction of radioactivity expired as TBA was low (~4%) during the first 3 hr, but increased over the next 3 hr such that it accounted for 37–42% of the expired radioactivity during this time period. In the high dose group, TBA accounted for a lesser percentage of expired radioactivity: ~1% during the first 3 hr and ~10% during the subsequent 3 hr.

In summary, the toxicokinetic handling of MTBE is quite similar following both oral and inhalation exposures. This similarity suggests that it is appropriate to use route-to-route extrapolation to establish a dose–response relationship for MTBE.

4. COMPARISON OF CRITICAL EFFECTS AMONG ROUTES

Only one oral study of MTBE has been performed in laboratory animals: a 90-day gavage in corn oil study in rats at doses of 0, 100, 300, 900, or 1200 mg/kg.[22] At the highest dose, profound anesthesia resulted, but was entirely reversible within a couple of hours. Decreased body weight gain was dose-related, but statistically significant only at the 1200 mg/kg. Other effects observed included decreased blood urea nitrogen (BUN) and serum creatinine, increased serum cholesterol, and loose stools at all doses; increased kidney weight at ≥300 mg/kg; and increases in other organ weights at ≥900 mg/kg. At the highest dose, changes in blood parameters and degenerative changes in the kidneys of male rats were reported. Although there were questionable effects at the 100 mg/kg dose level, there were more clearly adverse effects at 300 mg/kg. The critical target organs in this study appeared to be the kidney and liver, based on organ weight changes and clinical chemistry parameters at ≥300 mg/kg.

Dodd and Kintigh[23] administered MTBE to Fischer 344 rats (25/sex/group) for 13 wk (6 hr/d, 5 d/wk) at concentrations of 0, 800, 4000, or 8000 ppm. Ataxia occurred immediately following exposure for all of the rats in the high dose group. Slight hematological changes were noted at ≥4000 ppm. Both sexes in the high dose group had significantly increased cortisone levels, but no other biochemical findings were significant. Effects on organ weights included a concentration-related increase in the relative weights of liver, kidney, and adrenals in males and females (significant at ≥4000 ppm) and decreased absolute brain weight in males and females at 8000 ppm. Neurobehavioral endpoints showed sporadic effects at 4000 and 8000 ppm.

Burleigh-Flayer *et al.*[24] conducted a chronic inhalation study in which CD- 1 mice were exposed to 0, 400, 3000, or 8000 ppm MTBE for 18 months (6 hr/d, 5 d/wk). The lowest concentration of 400 ppm was considered to be a NOAEL and 3000 ppm was considered to be a LOAEL based on clinical chemistry effects and organ weight changes (particularly liver and kidney). The same laboratory conducted a 2-year study in F344 rats exposed to the same regimen described for the mice.[25] The rats were more sensitive to the effects of MTBE than the mice exposed to the same concentrations. Dose-related increases were observed for both rel-

ative and absolute kidney and liver weights in female rats exposed to 3000 or 8000 ppm. Clinical signs of CNS depression were also observed at the two highest doses. The lowest concentration of 400 ppm did not result in any adverse effects in female rats, but did result in an increased incidence and/or severity of progressive nephropathy in the male rats.

5. ROUTE-TO-ROUTE EXTRAPOLATION

The available toxicokinetic data permit a comparative quantitative estimation of absorption, distribution, metabolism, and excretion for the routes of interest. Previous discussion indicates that the critical noncancer effects (i.e., kidney and liver toxicity) are the same or closely related between the inhalation and oral routes of exposure. Finally, adequate toxicity are available for the inhalation route of exposure to be used as a basis for extrapolation to the oral route of exposure.[6,7] These criteria being fulfilled permits the estimation of roughly comparable oral doses given an inhaled concentration by way of the following equation:

$$\text{Oral Dose (mg/kg-day)} = \text{Inhaled Dose (mg/kg-day)} \times [\text{ratios of: absorption (I/O)} \times \text{distribution (I/O)} \times \text{metabolism (I/O)} \times \text{excretion (I/O)}] \quad (2)$$

In this equation, inhaled dose is a function of both the type of chemical (particle or gas) and the location of the critical effect (whether pulmonary or systemic) using the definitions found in EPA (10) for the determinations of Reference Concentration (RfC). MTBE is considered a gas and its critical effect is on the extrarespiratory system. Thus, the equation to use for calculating the inhaled dose is adapted from EPA (10) as:

$$\text{Inhaled Dose (mg/kg-day)} = \text{NOAEL}_{\text{ADJ}} \times \text{VR} \div \text{bw} \quad (3)$$

where $\text{NOAEL}_{\text{ADJ}}$ = animal concentration adjusted to reflect a continuous exposure (mg/m³), VR[3] = alveolar ventilation rate (m³/d), bw = animal body weight (kg). Combining this with Eq. (2) we find:

$$\text{Oral Dose (mg/kg/day)} = \text{NOAEL}_{\text{ADJ}} \times \text{VR} \div \text{bw} \times [\text{ratios of: absorption (I/O)} \times \text{distribution (I/O)} \times \text{metabolism (I/O)} \times \text{excretion (I/O)}] \quad (4)$$

For absorption, MTBE is nearly completely absorbed following oral exposure, less so than for inhalation, with estimates of near 100% for oral exposure and ranging about 40% to less than 100% for inhalation exposure. Therefore, the ratio of absorption (I/O) could be anywhere from about 0.4 to 1. For this analysis we use 0.5. However, other choices are possible and they could make up to a twofold difference in the outcome that we present. For each of the other I/O ratios, a value of 1 is used because the toxicokinetic studies suggest similar distribution, metabolism, and excretion of MTBE following inhalation and oral exposures at levels that do not produce saturation of enzyme kinetics.

There are sufficient toxicokinetic data to support a route-to-route extrapolation of toxicity data from the inhalation route of exposure to the oral route for MTBE. Also, the subchronic oral gavage study of Robinson *et al.*[22] with Sprague Dawley rats and subchronic inhalation study of Dodd and Kintigh[14] with F344 rats are suitable for use in comparing toxicity by these two routes. In particular, the NOAEL and LOAEL concentrations of the inhalation study will be used to estimate the equivalent doses for kidney weight increases from the oral study, since relative and absolute kidney weight increases were the most sensitive parameters monitored in both studies.[5]

From Dodd and Kintigh[23] we find that statistically significant ($p \leq 0.01$) increases in the absolute and relative weights of the kidneys were observed in both males and females at 4000 (14,400 mg/m³) and 8000 ppm (28,800 mg/m³). The NOAEL for this effect was 800 ppm (2880 mg/m³). Adjusting the NOAEL and LOAEL for an exposure regimen of 6 hr/d, 5 d per wk to yield an equivalent concentration for continuous exposure results in a $\text{NOAEL}_{\text{ADJ}}$ of 510 mg/m³ and a $\text{LOAEL}_{\text{ADJ}}$ of 2600 mg/m³. The equivalent oral NOAEL dose in F344 rats (with assumed average body weight of 0.25 kg) can be estimated from this exposure by:

$$\text{Oral NOAEL Dose (mg/kg-d)} = \text{NOAEL}_{\text{ADJ}} \times \text{VR} \div \text{bw} \times [\text{ratios of: absorption (I/O)} \times \text{distribution (I/O)} \times \text{metabolism (I/O)} \times \text{excretion (I/O)}]$$

or

$$\text{Oral NOAEL Dose (mg/kg-day)} = \text{NOAEL}_{\text{ADJ}} \times \text{VR} \div \text{bw} \times [\text{ratios of: absorption (I/O)} \times \text{distribution (I/O)} \times \text{metabolism (I/O)} \times \text{excretion (I/O)}]$$

[4] The ventilation rate is determined based on allometric equations presented in EPA (26). For rats, the equation is $\text{VR} = 0.80\text{W}^{0.8206}$. For mice, the equation is $\text{VR} = 1.99\text{W}^{1.0496}$.

[5] A benchmark dose approach to this comparison would be preferred since a consistent level of toxic effect could be compared between these two studies. Future work may wish to include the development of benchmark doses.

Table II. Comparison of Oral Doses of the Robinson *et al.* (22) Study and Equivalent Oral Doses from Dodd and Kintigh (23) as a test of Eq. (5)

	Relative kidney weight increase		Absolute kidney weight increase	
Effect	Robinson *et al.* (23) (actual)[a]	Dodd and Kintigh (24) (estimated)[b]	Robinson *et al.* (23) (actual)[a]	Dodd and Kintigh (24) (estimated)[b]
NOAEL mg/kg/day	100 females, 300 males	270	1200 females, 300 males	270
LOAEL mg/kg/day	300 females, 900 males	1400	> 1200 females, 900 males	1400

[a] Statistically significant at the $p \leq 0.05$ level in Sprague-Dawley rats.
[b] Statistically significant at the $p \leq 0.01$ level in F344 rats.

Similarly, the equivalent oral LOAEL dose in F344 rats can be estimated by:

$$\text{Oral NOAEL Dose} = 510\ \text{mg/m}^3 \times 0.26\ \text{m}^3\text{/day} \div 0.25\ \text{kg} \times (0.5 \cdot 1 \cdot 1 \cdot 1) \approx 270\ \text{mg/kg-day}$$

A comparison of these values is shown in Table II.

5.1. Confidence in the Route-to-Route Extrapolation

Given that limited and conflicting information exists on the oral toxicity of MTBE,[6,7] the decision to extrapolate an estimate of oral toxicity for MTBE from inhalation data depends on whether such an extrapolation increases our overall confidence in the final risk estimate. With MTBE, increased confidence in such an extrapolation appears justified. The inhalation data base is extensive, with chronic studies in two species, reproductive and developmental bioassays, and special studies. At least with noncancer toxicity, the critical effects, kidney and liver toxicity, appear to be independent of route of exposure. The available information on toxicokinetics shows similarities in distribution, metabolism and excretion, and a possible difference in absorption (ratios of inhalation to oral absorption range from 0.4 to 1).

MTBE has been evaluated for human health toxicity under the Canadian Environmental Protection Act.[28] Calculation of a Tolerable Daily Intake (TDI) was performed using the inhalation NOAEL[6] of 2915 mg/m^3 identified by Dodd and Kintigh.[23] Extrapolation of this inhalation concentration to an oral exposure was performed by assuming an inhalation rate of 0.144 m^3/day for adult rats. Factoring in exposure conditions of 6 hr/d, 5 d/wk and a body weight of 0.25 kg resulted in an equivalent oral NOAEL of 300 mg/kg-d. It is noted that Health Canada only conducts route-to-route extrapolation in exceptional cases.[28]

The use of the equation proposed in this report results in estimated oral NOAEL values for relative kidney weight increase in F344 rats of 270 mg/kg-d (from Table II), based on the inhalation study of Dodd and Kintigh.[23] These estimates are very close to those proposed by Long *et al.*[27] Furthermore, these estimated oral NOAELs are roughly the same as the actual oral NOAELs[7] of 100 or 300 mg/kg-d (females and males, respectively) in the Robinson *et al.*[22] study for relative kidney weight increase using Sprague Dawley rats. Similarity also exists for the estimated oral NOAEL for absolute kidney weight increase when compared to the actual NOAEL of Robinson *et al.*[22] for males (300 vs. 270 mg/kg-d). There is some difference between the estimated LOAELs when compared to the actual LOAELs for both relative and absolute kidney weights, but the values are comparable, with most being within a fivefold factor. This is not unexpected, as differences may result from a number of factors, such as possible differences between strains of rats, and toxicokinetic effects related to gavage administration at higher doses. It may also be a reflection of the limitations imposed by the investigators' choice of doses, to which the NOAEL and LOAELs are constrained. A benchmark dose analysis would be useful in resolving this potential uncertainty since the form of the existing data cannot resolve it.

EPA[30] has estimated a Reference Concentration (RfC) for noncancer toxicity of MTBE on the basis of the 2-year rat bioassay of Chun *et al.*,[24] but does not have a cancer risk assessment. EPA's RfC of 3 mg/m^3 is based on an adjusted NOAEL of 259 mg/m^3. Other concentrations in the study included an adjusted LOAEL

[6] It is noted by Environment Canada that while this concentration was identified as a NOAEL, it was associated with increases in relative kidney and liver weights (not statistically significant) in male rats and might therefore be more appropriately considered to be a LOAEL.

[7] While the dose of 100 mg/kg/day is cited as a NOAEL for the Robinson et al study, it is noted that increased serum cholesterol and diarrhea were observed at all doses, leading others to conclude that this dose is more appropriately considered a LOAEL (29).

of 1946 mg/m³ and a high dose of 5136 mg/m³. Equation (4) can be used with the concentrations of the 2-year rat bioassay of Chun *et al.*[24] to determine equivalent oral doses. Using an average ventilation rate of 0.33 mg/m³ and an average body weight of 0.34 kg for adult F344 rats results in equivalent oral doses of about 130, 940, and 2700 mg/kg-d for the low (259 mg/m³), middle (1946 mg/m³) and high concentrations (5136 mg/m³), respectively, of the Chun *et al.*[24] study.[8] These equivalent doses may be useful in conducting noncancer and cancer risk assessments for the oral route of exposure based on the results found from inhalation.

ACKNOWLEDGMENTS

We wish to thank Drs. Nancy Balter and James Embree for their very helpful comments during the development of this manuscript. Dr. Balter's insights in particular assisted in the full explication of toxicokinetic data we display. Dr. Embree provided valuable clarifications to the ideas we discuss.

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