Appendix D

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Application of a Physiologically-Based Pharmacokinetic Model for Reference Dose and Reference Concentration Estimation for Acetone

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Running Title: Acetone RfD/RfC Using a PBPK Model

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ABSTRACT

Recent health risk assessments to propose a Reference Dose (RfD) for acetone (Forsyth 2001; USEPA 2001) have been based on the results of an oral subchronic study conducted in rats and mice (Dietz et al. 1991; NTP 1991). These assessments have utilized the traditional concept of establishing the RfD by determining the lowest experimentally determined No-Observed-Adverse-Effect Level (NOAEL) and applying various uncertainty factors (USEPA 1988). This paper describes a risk assessment for acetone based on the systemic toxicity observed in subchronic and developmental toxicity studies to estimate an RfD and an inhalation Reference Concentration (RfC) for acetone. Specifically, this approach examined the subchronic study by Dietz et al. (1991), as well as an inhalation developmental toxicity study on acetone (Mast et al. 1988) and several toxicology studies of isopropanol (IPA). This was accomplished by applying a physiologically based pharmacokinetic (PBPK) model developed previously for isopropanol (IPA) and its metabolite acetone (Clewell et al. 2001). The incorporation of the PBPK model into the derivation of an RfD and RfC for acetone allowed for a tissue-based approach rather than an external exposure based approach, making it possible to derive an oral RfD from an inhalation study. In addition, the use of the PBPK model to analyze data from chronic and reproductive/developmental studies conducted with IPA enabled an assessment of the potential for acetone to produce any of the effects observed in the IPA studies. This analysis provided sufficient information to reduce the need for Uncertainty Factors in the adjustment of the NOAEL from the oral subchronic study for the determination of an RfD. Using the PBPK model in the acetone risk assessment supports a composite Uncertainty Factor of 60 for the subchronic study, compared to composite factors of 300 to 3000 in the other recent risk assessments. This difference resulted in an RfD of 16 mg/kg/day, compared to the values of 0.3 to 3 that have

previously been estimated (Forsyth 2001; USEPA 2001). Considering the results from the inhalation developmental study (Mast et al. 1988) resulted in an RfD of 8.7 mg/kg/day. Using this study also fills a data gap for acetone that exists if only the oral database for acetone is considered for RfD derivation. An RfC of 29 ppm was also estimated for acetone using the Mast et al. (1988) study results in combination with the PBPK model. The potential impact of endogenous acetone on a risk assessment for acetone is also discussed.

keywords: PBPK model, Reference Dose, RfD, Reference Concentration, RfC, acetone, risk assessment, CAS# 67-64-1

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model has been developed for isopropanol (IPA) for both the rat and human (Clewell et al. 2001). This model has been applied to perform route-to-route and cross-species dosimetry in support of a risk assessment for IPA (Gentry et al. 2002). This model also contains a complete PBPK submodel for the major IPA metabolite, acetone, which can be used to describe pharmacokinetics following acetone exposure. This submodel has been validated by showing that is simulated the available acetone pharmacokinetic data from studies conducted in both rats and human subjects (Clewell et al. 2001).

The U. S. Environmental Protection Agency (USEPA) is currently revising the Reference Dose (RfD) for acetone, based on the results of a subchronic drinking water study conducted in rats and mice (Dietz et al. 1991; NTP 1991). An RfD has also recently been developed by Forsyth (2001), based on the same studies using a traditional approach of estimating an RfD based on the lowest NOAEL from mammalian toxicity studies. Therefore, neither of these risk assessments made use of a PBPK model to conduct route-to-route or cross-species extrapolation. With the increasing trend in the incorporation of PBPK models into chemical risk assessment and the availability of a validated model for acetone, it was decided to investigate the potential impact of the incorporation of a PBPK model into an acetone risk assessment.

In the risk assessment conducted by Forsyth (2001), a NOAEL of 900 mg/kg/day reported in the Dietz et al. (1991) study was used in combination with a total Uncertainty Factor (UF) of 300: 10 for human variability, 10 for subchronic to chronic extrapolation, and 3 for interspecies extrapolation, resulting in an RfD of 3 mg/kg/day. In the proposed risk assessment

conducted by the USEPA (2001), a larger UF of 3000 was used, including an additional factor of 10 for the lack of a complete dataset for reproductive and developmental effects, and for lack of a chronic study, resulting in an RfD of 0.3 mg/kg/day.

The purpose of the work described in this paper is to demonstrate the use of a validated acetone PBPK model in the development of both an RfD and RfC. For the estimation of the RfD, two approaches were used. The first approach involved the use of the available oral dataset for acetone and focused on the same study that is the current basis for the proposed RfD (Dietz et al. 1991; NTP 1991). In addition to using the PBPK model to estimate the most appropriate internal dose metric associated with the NOAEL, the model was also used to evaluate the appropriateness of Uncertainty Factors applied in standard risk assessments. An Uncertainty Factor has been considered in the estimation of the proposed RfD for lack of a complete dataset for acetone; however, the database for IPA, whose major metabolite is acetone, is considered complete. The PBPK model, validated for both acetone and IPA, was therefore used, in combination with the available studies for IPA, to address the necessity of this Uncertainty Factor in this risk assessment for acetone.

The second approach involved the use of an inhalation developmental study for acetone (Mast et al. 1988), which has not been considered in the USEPA's estimation of the RfD because it was not conducted via the oral route of exposure. Current default approaches for RfD derivation provide no guidelines for the use of toxicity data from a route of exposure other than the oral route. One advantage of the incorporation of validated PBPK models into the risk assessment process is that these models can be used to perform cross-species and cross-route extrapolations. The acetone rat PBPK model can be used to estimate the target tissue dose

associated with the NOAEL in a toxicity study conducted by a given route of administration. The human PBPK model subsequently enables the prediction of the equivalent human exposure by another route that would result in the same target tissue dose. This type of cross-route comparison using a PBPK model has been conducted by the USEPA (2000) in the development of an RfC for vinyl chloride, which is based on data from an oral study. This approach to the estimation of an RfD was considered not only because it can be conducted using a PBPK model, but also because in the risk assessment for IPA (Gentry et al. 2002), the lowest NOAEL established following either oral or inhalation exposure was reported in developmental studies (Tyl et al. 1994).

METHODS

The approaches used for the derivation of the RfD and RfC for acetone followed the general approach used by the USEPA (1988; 1994), with the use of the previously developed rat and human PBPK models incorporated into the process. The IPA/acetone adult models for both the human and the rat have been described previously (Clewell *et al*., 2001), as well as the extension of the models to simulate exposure in either rats or humans during pregnancy, in order to provide estimation of internal dose metrics for the fetus during developmental studies (Gentry et al., 2002). The basic structure of the IPA and acetone models is provided in Figures 1 and 2. The appropriate rat PBPK model (adult or pregnancy) was used to estimate the internal dose metric that was associated with the NOAEL and that was most relevant to the endpoint observed. Once the internal dose metric associated with the NOAEL was estimated, the appropriate UFs were applied to estimate the corresponding human internal dose metric. The human PBPK

model was then used to estimate the oral or inhalation exposure that would result in the human internal dose metric.

Summary of Critical Studies

Two studies were considered to provide the basis for the derivation of an RfD for acetone: a subchronic drinking water study (Dietz et al. 1991; NTP 1991) and an inhalation developmental study (Mast et al. 1988). In the Dietz et al. (1991) study, groups of 10 F344/N rats/sex, as well as groups of 10 female mice were administered drinking water containing 0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm acetone, while male mice were administered drinking water containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm acetone. The majority of the effects observed following acetone administration were observed in male rats. A variety of mild and subtle hematological changes (i.e., increased leukocyte count, mean corpuscular hemoglobin, and mean cell volume; decreased erythrocyte, platelet, and reticulocyte count) were observed in males administered 20,000 or 50,000 ppm. These changes were consistent with mild reactive macrocytic normochromic anemia with a depressed regenerative response (reticulocytes). Acceleration in the occurrence and severity of spontaneously occurring nephropathy, a progressive condition seen in the kidney of aging rats, was also observed in male rats administered 20,000 or 50,000 ppm acetone. Based on these effects, a No Observed Adverse Effect Level (NOAEL) of 10,000 ppm (900 mg/kg/day) was estimated for the derivation of an RfD.

In the Mast et al. (1988) study, groups of 30-33 presumed pregnant Sprague-Dawley rats and Swiss CD-1 mice were exposed to acetone by inhalation for 6 hours per day, 7 days per

week from gestation day 6 to 17 (mice) or 19 (rats). Rats were exposed to 0, 440, 2,200, or 11,000 ppm acetone vapors, while mice were exposed to 0, 440, 2,200, or 6,600 ppm acetone vapors. The statistically significant effects observed in rats following exposure were limited to the high concentration group and included decreases in body weight, uterine weight, and extragestational weight in the dams, and decreases in fetal weight. The incidence of any single fetal malformations was not significantly increased at any exposure concentration. However, by combining all types of malformations, the percent of litters containing at least one pup with a malformation was 11.5% in the high-concentration group (3/26), compared to 3.8% in the control group (1/26). In mice, a slight but statistically significant decrease in fetal weight was also observed in the high concentration group, as well as a significant increase in the incidence of late resorptions and increases in maternal liver to body weight ratios. These results indicate that *in vivo* exposure to acetone during pregnancy was not teratogenic in either species and are consistent with the results of *in vitro* studies of acetone in rat embryo cultures (Kitchin and Ebron 1984) and in the mouse embryo limb bud assay (Guntakatta et al. 1984). Based on the effects on maternal/fetal body weight, the No Observed Adverse Effect Level (NOAEL) for developmental toxicity for both species was determined to be 2,200 ppm. The slope of the dose-response curve for maternal/fetal effects in the studies of Mast et al. (1988) was very shallow. That is, a three to five fold increase in exposure concentration from the middle (2,200 ppm) to the highest (6,600 or 11,000 ppm) concentration produced only a 15% reduction in fetal body weight in rats. Thus, it is likely that a NOAEL greater than 2,200 ppm could have been defined, it another exposure level between the highest concentration and the mid concentration had been added to these studies.

Estimation of the Internal Dose Metric Associated with each Animal NOAEL

For the estimation of the internal dose metrics associated with the NOAEL reported by Dietz et al. (1991), the rat adult acetone PBPK model was used. Simulations using the adult rat model were conducted for either the duration of exposure or until steady-state or periodicity had been reached. At the end of each simulation, the average daily area under the blood concentration curve (AUC) for acetone was calculated.

The NOAEL reported in the Mast et al. (1988) study is based on fetal body weight effects observed in rats. Therefore, the rat pregnancy model (Gentry et al. 2002) was used to simulate the study-specific exposure on gestation day 6 to 17 in order to estimate the internal tissue dose of acetone to the fetal compartment. An AUC was then calculated as the total area under the fetal concentration-time curve for acetone, divided by the length of exposure during pregnancy.

Consideration of Uncertainty Factors

Once the internal dose metric associated with each NOAEL was estimated, the composite UF was applied to estimate the desired human internal dose metric. In determining the composite UF for each RfD estimate, the five categories of UFs applied by the USEPA (1988; 1994) in noncancer risk assessment were considered. These include a factor of up to 10 for use of a subchronic instead of a chronic study, a factor of up to 10 for use of a LOAEL instead of a NOAEL, a factor of up to 10 for interspecies variability, a factor of up to 10 for intraspecies variability and a factor of up to 10 for incomplete data base. As discussed previously, a UF for database insufficiency has been suggested for the derivation of an acetone RfD, due to the lack

of a complete dataset for reproductive and developmental effects and lack of a chronic study. However, the dataset for IPA, whose major metabolite is acetone, is complete. One of the goals of this effort was to determine whether this UF might not be necessary if the results from the available chronic and reproductive/developmental studies for IPA were considered in the derivation of the RfD for acetone.

Using the IPA/acetone PBPK model, three internal dose metrics associated with the IPA exposure concentrations or doses administered in the chronic and reproductive/developmental studies were estimated. Specifically, the internal dose metrics were: the average daily area under the blood IPA concentration curve (AUC_{IPA}), the average daily area under the blood acetone concentration curve (AUC_{ace}), and the average daily area under the IPA and acetone blood concentration curves combined (AUC_{com}). A comparison of the toxicity observed in the subchronic studies for IPA and acetone was made, along with a comparison of the estimated internal dose metrics for each study, to evaluate which dose metric appeared to be most appropriate for comparison across studies. The dose metric judged to be most appropriate from the IPA studies was then compared to the internal dose metric associated with the NOAEL from the Dietz et al. (1991; NTP 1991) study to determine if an RfD estimated based on the subchronic study would be health protective for potential reproductive/developmental or chronic effects resulting from exposure to acetone, or whether an additional UF was needed. A comparison of the dose metrics from the IPA reproductive/developmental studies with the internal dose metric associated with the NOAEL from the acetone developmental study was also performed to see if the dose metrics associated with the developmental studies across chemicals were consistent.

Derivation of the RfD Using the Human PBPK Model

Once the appropriate composite UF was applied to the internal dose metric estimated with the rat PBPK model, the human version of the model was then used to estimate the oral exposure associated with the desired human internal dose metric. For the systemic endpoint reported by Dietz et al. (1991), this was done by adjusting the external concentrations in the human model until the human average daily AUC matched the rat internal dose metrics that had been adjusted by the appropriate composite UF.

For the developmental endpoint, an iterative two-step process was required using the human pregnancy model. First, the human adult model was run for continuous inhalation exposure at a given concentration until steady-state was reached. The final concentrations in the various tissues were estimated and used as initial tissue concentrations for the pregnancy model. The pregnancy model was then used to simulate a continuous exposure concentration, using the given initial tissue concentrations, for the duration of pregnancy. The daily AUC was calculated by dividing the total AUC during pregnancy by the length of pregnancy in days. As with the systemic endpoints, external concentrations were adjusted until the human average daily AUC matched the adjusted rat internal dose metrics.

RESULTS

Determination of the Internal Dose Metric for RfD Development

Application of the PBPK model requires selection of the appropriate dose metric, depending on the chemical mechanism of toxicity, or mode of action. The effects observed in

both the Dietz et al. (1991) study (i.e., hematological effects, renal effects) and the Mast et al. (1988) study (i.e., decreased fetal weights) would appear to be cumulative in nature, depending on both the concentration and duration of the exposure. The area under the concentration curve (AUC) is a simple metric for such cases, which is defined mathematically as the integral of the concentration over time (Andersen et al., 1987). Therefore, the AUC in the blood for the subchronic/chronic studies or the AUC in the fetus for the developmental toxicity studies was selected as the dose metric for the determination of the RfD and RfC for acetone. The AUCs for each of the studies under consideration for the estimation of an RfD for acetone are reported in Table 1.

Consideration of Uncertainty Factors

For the first approach, which relied upon the NOAEL reported in the subchronic drinking water study (Dietz et al. 1991), an evaluation of the UFs applied in the two draft risk assessments was considered. The two recent derivations of an RfD for acetone (Forsyth 2001; USEPA 2001) which both rely on the results of the Dietz et al. (1991) study, have applied composite UFs of 300 to 3000 as adjustments to the study-derived NOAEL. The first factor considered in the estimation of the RfD using the PBPK model was a factor of 10 for human variability. This factor is still applicable, even with the use of the PBPK model, in estimating an RfD from the NOAEL reported by Dietz et al. (1991), because it accounts for potential responses from the more sensitive members of the population.

In both of the draft assessments of an RfD, a partial UF of 3 ($10^{1/2}$) was added for interspecies extrapolation to account for uncertainties regarding differences in pharmacokinetics

between animals and humans. The factor of 10 generally considered for interspecies extrapolation is commonly taken to consist of a partial factor of 3 for pharmacokinetics and a partial factor of 3 for pharmacodynamics. However, in both of the draft assessments it was judged that the lesions observed following acetone exposure were not considered to adversely affect the health of the animals clinically (no clinical chemistries changes associated with the renal effects observed and possible relation to hyaline droplets and only mild anemia). In addition, the renal effects were of questionable biological significance to humans, so that a partial factor for pharmacodynamic uncertainties was considered unnecessary. With the incorporation of a PBPK model into the risk assessment process, the partial factor of 3 for pharmacokinetic uncertainties is no longer necessary. The PBPK models for the rat and the human incorporate the available quantitative information on the pharmacokinetics in each species, eliminating the need for adjustments with an UF. Thus, no UF for interspecies extrapolation is needed for the RfD.

An additional factor of 10 was applied by the USEPA (2001) in consideration of limitations in the database for acetone, which is lacking in the areas of reproductive/developmental and chronic studies. However, these two types of studies have been conducted for IPA. In the derivation of an RfD/RfC for IPA (Gentry et al. 2002), the results of five studies were considered: two subchronic inhalation toxicity studies with behavioral endpoints (Burleigh-Flayer et al.,1994, 1998), an inhalation developmental study (Nelson et al. 1988), an oral developmental toxicity study, including neurotoxicity (Tyl et al., 1994), an oral two-generation reproductive toxicity study (Bevan et al., 1995), and a chronic inhalation toxicity/oncogenicity study (Burleigh-Flayer et al., 1997). For the systemic effects, the three

difference AUCs were estimated for the adult, while for the developmental effects, AUCs were estimated for the fetus.

In order to compare the results from the IPA chronic and reproductive/developmental studies to the results from the subchronic study for acetone, the subchronic studies for IPA and acetone were compared to determine the internal dose metric that would be most appropriate for the comparison. In the IPA subchronic study (Burleigh-Flayer et al. 1994), blood changes (i.e., decreased total erythrocytes, hemoglobin, hematocrit and platelet counts, increased MCV and MCH) observed in male and female rats exposed to 5000 ppm IPA via inhalation (the highest concentration tested) were similar to those in the acetone subchronic study following drinking water exposure to 20,000 ppm. However, the changes in the IPA study were transient, with significant changes in treated animals, compared to control animals, observed following six weeks of exposure, but with no significant change in many of the parameters remaining after 14 weeks of exposure. Renal effects related to acceleration of age-related kidney nephropathy were also observed in both studies, but were more advanced in male rats following exposure to 20,000 ppm acetone in drinking water.

Since acetone is the primary metabolite for IPA, a complicating factor in the selection of a dose metric for comparison across acetone and IPA studies is the determination of relative toxicological equivalence of the two substances. Specifically, in the IPA studies, what was the toxicologically active form of the chemical (*i.e.*, IPA, acetone, or a combination of the two).

A comparison of the AUCs associated with the NOAELs/LOAELs from the subchronic studies for IPA and acetone are provided in Table 2. Slight changes in blood parameters (i.e., increased mean cell volume and mean corpuscular hemoglobin) were observed following both

inhalation exposure to IPA (5000 ppm) in the Burleigh-Flayer et al. (1994) study and oral exposure to acetone (900 mg/kg/day) in the Dietz et al. (1991) study. Because the profile of hematological effects observed with these two exposure groups is very similar, it is expected that the internal dose metrics associated with these two exposure group would be similar. As the results in Table 2 indicate, the estimated internal dose metric for IPA and acetone combined from the Burleigh-Flayer et al. (1994) study (10709 mg-hr/L) is very similar to the internal dose metric for acetone only estimated for the Dietz et al. (1991) study (10440 mg-hr/L) . This supports the assumption that both IPA and acetone were similar in their mode of action and potency for the production of these mild hematological effects in rats. The internal dose of acetone estimated following inhalation of 5000 ppm of IPA (AUC_{ace}=7047 mg-hr/L) was clearly below the internal dose required for the production of hematological effects in the study reported by Dietz et al. (1991) (AUC_{ace}=22,514 mg-hr/L). These results indicate that the formation of acetone as a metabolite of IPA was not the solely active material for the elicitation of hematological effects observed in the IPA study. In addition, they provide support that neither synergism nor inhibition occurred between IPA and its metabolite acetone in the toxicity studies of IPA. These results indicate that the valid comparison across studies would be the combined dose metric from the IPA studies for comparison to the internal dose metric from the acetone studies.

The AUCs estimated for each of the IPA chronic and reproductive/developmental studies (Gentry et al. 2002) is provided in Table 3. If the acetone internal dose metric associated with the NOAEL from Dietz et al. (1991) (10440 mg-hr/L) is compared with the appropriate AUC_{com} estimated from the IPA studies, it is clear that the internal dose metric associated with the

NOAEL from the Dietz et al. (1991) study is greater than those associated with the IPA studies (Table 3). The difference between the AUC_{ace} from the Dietz et al. (1991) study and the AUC_{com} from the IPA chronic study (Burleigh-Flayer et al. 1997) is approximately a factor of 2 (10440 versus 4581 mg-hr/L). The largest difference between AUCs for acetone and IPA is between the AUC from the subchronic Dietz et al. (1991) study and the AUC from the developmental study conducted by Tyl et al. (1994) (10440 versus 1750 mg-hr/L). This difference is approximately a factor of 6, which would indicate that an adjustment of the acetone NOAEL from Dietz et al. (1991) by a factor of 6 should be health protective of reproductive/developmental effects and also chronic effects that may results from exposure to acetone, rather than a factor of 10.

An uncertainty factor of 10 has also been applied in both previous assessments to extrapolate from subchronic to chronic exposure. However, based on the estimated daily average AUC for the NOAEL from the chronic IPA study (Burleigh-Flayer et al. 1997), which is only a factor of 2 lower than the subchronic studies, the uncertainty factor of 6 applied to adjust for database would also be protective of effects from chronic exposure to acetone. Therefore, the factor of 10 for uncertainties between subchronic and chronic exposure is not necessary.

In summary, consideration of the available information for acetone together with the available information for IPA using the PBPK model results in a reduction in the UFs necessary in the derivation of an RfD for acetone based on the Dietz et al. (1991) study. The resulting composite UF is 60, a factor of 6 for database insufficiency and a factor of 10 for human variability.

To derive an RfD based on the NOAEL from the inhalation developmental study conducted by Mast et al. (1988), those factors applied in the estimation of the RfD using the

Dietz et al. (1988) study were considered. The first factor applied in the derivation of the RfD was a factor of 10 for human variability. This factor is still applicable to the Mast et al. (1988) results, because it accounts for potential responses from the more sensitive members of the population. The second factor of 6 applied to the internal dose metric associated with the NOAEL from the Dietz et al. (1991) study was for database insufficiency. This factor of 6 was based on the difference between the AUC associated with the NOAEL in Dietz et al. (1991) and the AUC from the developmental study for IPA conducted by Tyl et al. (1994). The internal dose metric associated with the NOAEL from the Mast et al. (1988) study (2664 mg-hr/L) is consistent with the internal dose metrics estimated for the IPA developmental studies (Table 3). It is also lower than the internal dose metric associated with the NOAEL from the IPA chronic toxicity study (Burleigh-Flayer et al. 1997). Therefore, this factor of 6 is no longer necessary if the RfD is estimated based on the NOAEL from the Mast et al. (1988) study. An uncertainty factor of 3 ($\sqrt{10}$) should be applied to account for uncertainties in pharmacodynamics across species. This factor was not applied in the estimation of the RfD based on the Dietz et al. (1991) study. This was because the lesions observed in the subchronic study (Dietz et al. 1991) were not considered adverse and were of questionable biological significance to humans. Using the NOAEL from the Mast et al. (1988) study as the basis for the RfD, a composite UF of 30 would be applied: a factor of 3 for pharmacodynamic differences across species and a factor of 10 for human variability.

Estimation of an RfD Based on Both Critical Studies

The PBPK rat model for acetone was run to simulate exposure to acetone in the drinking

water at a dose of 900 mg/kg/day, the NOAEL based on the results reported by Dietz et al. (1991), and an AUC of 10440 mg-hr/L was estimated. This rat AUC was then adjusted by the UF of 60 and the human model was run in order to determine the continuous human exposures that would result in the desired internal dose metric values. This calculation was accomplished by running the model at a constant drinking water concentration until steady-state had been reached, and adjusting the concentration until the calculated daily AUC for the given internal dose metric matched the adjusted rat internal dose metric value. The estimated RfD for acetone using the PBPK model is 16 mg/kg/day, compared to the proposed USEPA RfD of 0.3 mg/kg/day.

To estimate the internal dose metric associated with the NOAEL in the rat from the Mast et al. (1988) study, the PBPK rat model for acetone was run to simulate exposure to 2200 ppm acetone vapor for 6 hours per day, 7 days per week for gestational days 6 to 19. An internal dose metric of 2664 mg-hr/L was estimated for the fetal AUC, which, as discussed previously, is consistent with the fetal AUC_{com} corresponding to the NOAELs reported for the IPA developmental studies (Table 3). This fetal AUC in the rat was then adjusted by the UF of 30, and the human model was run in order to determine the continuous human oral exposure that would result in the desired internal dose metric values. The estimated RfD for acetone based on the fetal AUC is 8.7 ppm.

The estimation of an RfD based on the developmental effects reported by Mast et al. (1988) results in a lower RfD than that based on the subchronic study conducted by Dietz et al. (1991). Therefore, use of the developmental study (Mast et al. 1988) for the estimation of the RfD for acetone would be health protective for the observed subchronic effects (Dietz et al.

1991). This is also consistent with the observed effects following exposure to IPA.

Estimation of an RfC based on Mast et al. (1988)

The results from the developmental study of Mast et al. (1988) were also used to estimate an RfC for acetone using the PBPK model. An RfC has not previously been developed for acetone. This value is based on the same internal dose metric as that for the RfD and with the same UFs applied, resulting in an RfC of 29 ppm.

DISCUSSION

Endogenous Production of Acetone

One important consideration in the estimation of an RfD for acetone that was not included in this assessment is the endogenous production of acetone. Acetone levels in the body at any given point in time are reflective not only of external exposure, but also of energy expenditure through free fatty acid utilization and acetoacetate production by the liver. Because of this, many normal and abnormal physiological states can increase the measured levels of endogenous acetone through the process of ketogenesis (SIAR 1998).

Endogenous levels of acetone have been reported to vary widely over a population (SIAR 1998). As part of the Third National Health and Nutrition Examination Survey (NHANES III, 2000), a Priority Toxicant Reference Range Study was conducted to assess the levels of common pesticides in urine and volatile organic compounds (VOCs) in blood of the U. S. population. Blood samples were collected from a group of 911 volunteers between the ages of 20-59 years old. The mean acetone concentration for the group was approximately 3.1 mg/L, with a standard

deviation of approximately 4.3 mg/L. The 95th percentile for this distribution was approximately 9 mg/L and the maximum was approximately 80 mg/L. Levels in children and adolescents may be even greater than in adults, due to their higher energy expenditure; levels reported in infants 2 to 5 days of age ranged as high as 140 mg/L (SIAR, 1998).

Plasma acetone concentrations associated with various human physiological states or conditions have been estimated (SIAR 1998). Plasma concentrations of less than 10 mg/L have been considered as "healthy levels" in individuals, while occupational exposures have been associated with plasma concentrations of <100 mg/L. Exposures classified as "toxic", were reported to be associated with plasma concentrations of >200 mg/L (SIAR 1998).

The use of the PBPK model for acetone to derive an RfD and RfC based on the NOAEL from the Mast et al. (1988) study, in combination with the UF factor of 30, results in an estimated plasma concentration of approximately 4 mg/L. This exposure-derived concentration would add to the endogenous levels of acetone, which are approximately 3.1 mg/L based on the results of the Priority Toxicant study of the NHANES III (1998) study. Therefore, average blood concentrations of approximately 7 mg/L would be expected to result from exposures associated with an RfD of 8.7 mg/kg/day. Due to the variation in endogenous acetone levels, total acetone blood concentrations following acetone exposure at the recommended RfD could range as high as approximately 13 mg/L, using the 95th percentile for the distribution of endogenous acetone levels reported in NHANES III (1998).

Thus the estimated total (exogenous plus endogenous) acetone plasma concentration following exposure to the recommended RfD is on the order of levels considered "healthy" in the SIAR (1998) report. The upper bound estimate of 13 mg/L is approximately an order of

magnitude lower than plasma concentrations that have been characterized in the SIAR (1998) as associated with toxicity.

Application of Tissue Dose Based Approaches

The current assessment demonstrates the strengths of a tissue dose based approach to the estimation of reference values. Standard default methods for the derivation of Reference Doses or Concentrations rely on the use of external exposure concentrations, when the most relevant measure of dose is that which reaches the target tissue. This target tissue dose is then assumed to result in the same responses across species, unless pharmacodynamic differences can be established. The use of PBPK models allows for the estimation of target tissue dose metrics incorporating all of the available quantitative chemical-specific pharmacokinetic information. This type of approach also removes the current practice of the route of exposure in the animal study being restricted to the relevant human exposure. Because the assessment is based on internal dose metrics, external concentrations or doses that are associated with a specific target tissue dose metric can be estimated with a human PBPK model regardless of the route of exposure in the animal study. This approach has been demonstrated for acetone by using an inhalation developmental study to estimate an oral RfD. The ability to consider the inhalation developmental study for acetone in the estimation of the RfD also fills the data need of a developmental study by the oral route that was accounted for by the USEPA (2001) through the use of UFs.

The current assessment used in the derivation of both an RfD for acetone has not only used a PBPK model for the estimation of a human equivalent concentration, but it has

demonstrated an approach for using a PBPK model's capability to compare the results of studies not only across routes of exposure, but also across metabolically-linked chemicals that have similar modes of action (i.e., acetone and IPA). This capability has been used to critically evaluate the UFs used in the most recent acetone risk assessments and determine the necessity of these factors, resulting, in the case of the RfD, in a decrease of 50 in the composite UF for the acetone risk assessment. In addition, the dose metrics from the acetone developmental study could be compared with those from multiple developmental studies conducted for IPA. The results of this comparison demonstrated consistency for similar types of studies across two chemicals. This type of comparison could be applied for other chemicals that share the same critical dose metric and could be used to fill data gaps in a similar manner.

In conclusion, this risk assessment for acetone has focused on the incorporation of an existing PBPK model for IPA, with a full submodel reflecting acetone pharmacokinetics, into the derivation of both an RfD and RfC for acetone. The model has been validated using the available acetone pharmacokinetic data in both animals and humans as part of the development of the PBPK model for IPA for use in an IPA risk assessment. In conducting a risk assessment for acetone, data were available to take advantage of the PBPK model in an innovative approach for evaluating the application of uncertainty factors in a noncancer risk assessment. Because IPA's major metabolite is acetone and the existing IPA PBPK model considered acetone pharmacokinetics, the existing database for IPA was used in conjunction with the available data for acetone to provide information on the potential for chronic or reproductive/developmental effects following exposure to the proposed RfD/RfC for acetone.

If the Dietz et al. (1991) study was used for the derivation of the RfD, the composite UF

decreased from 300 (Forsyth 2001) or 3000 (USEPA 2001) to 60, resulting in an RfD of 16 mg/kg/day. An RfD was also estimated based on the internal dose metric associated with the inhalation developmental study reported by Mast et al. (1988). This RfD was 8.7 mg/kg/day, about a factor of 2 lower than the RfD estimated based on the Dietz et al. (1988) study, but greater than the current RfD of 0.3 mg/kg/day reported by the USEPA (2001). These results indicate that the use of the inhalation developmental study would provide a more sensitive endpoint for the estimation of RfD and RfC, resulting in recommended values of 8.7 mg/kg/day and 29 ppm, respectively.

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Table 1
Rat Dose Metrics Associated with NOAELs Derived from Toxicity Studies for Acetone

Study Type/Study	Endpoint	NOAEL	Daily Area under the Arterial Blood Concentration Curve for Acetone (AUC_{ace}) (mg-hr/L)
Subchronic Study Dietz et al. (1991)	Hematological, renal effects	900 mg/kg/day	10440
Developmental Study Mast et al. (1988)	Decreased fetal body weights	2200 ppm	2664

Table 2
Comparison of Dose Metrics from Burleigh-Flayer et al. (1994) and Dietz et al. (1991)

Study	Endpoint		Daily AUC_{ace}	Daily AUC_{com}
Dietz et al. 1991	Mild Hematological Effects	900 mg/kg/day (NOAEL)	10440	--
		1700 mg/kg/day (LOAEL)	22514	--
Burleigh-Flayer et al. 1994	Mild Hematological Effects	5000 ppm (NOAEL/LOEL)	7047	10709

Table 3
Rat Dose Metrics Associated with NOAELs Derived from Toxicity Studies for IPA

Study Type/Study	Endpoint	NOAEL	Daily Area under the Arterial Blood Concentration Curve for IPA and Acetone Combined (mg-hr/L)
Chronic Studies Burleigh-Flayer et al. (1997)	Chronic renal disease	2500 ppm (females)	4581
Developmental Studies Nelson et al. (1988) Tyl et al. (1994) Bevan et al. (1995)	Decreased fetal body weight	3500 ppm 400 mg/kg/day 500 mg/kg/day	4784 1750 1932
Reproductive Studies Bevan et al. (1995)	Decreased male mating index	420 mg/kg/day[a]	3837

[a]Lower bound on dose associated with a 5% response rate estimated using benchmark modeling (Allen et al. 1998).

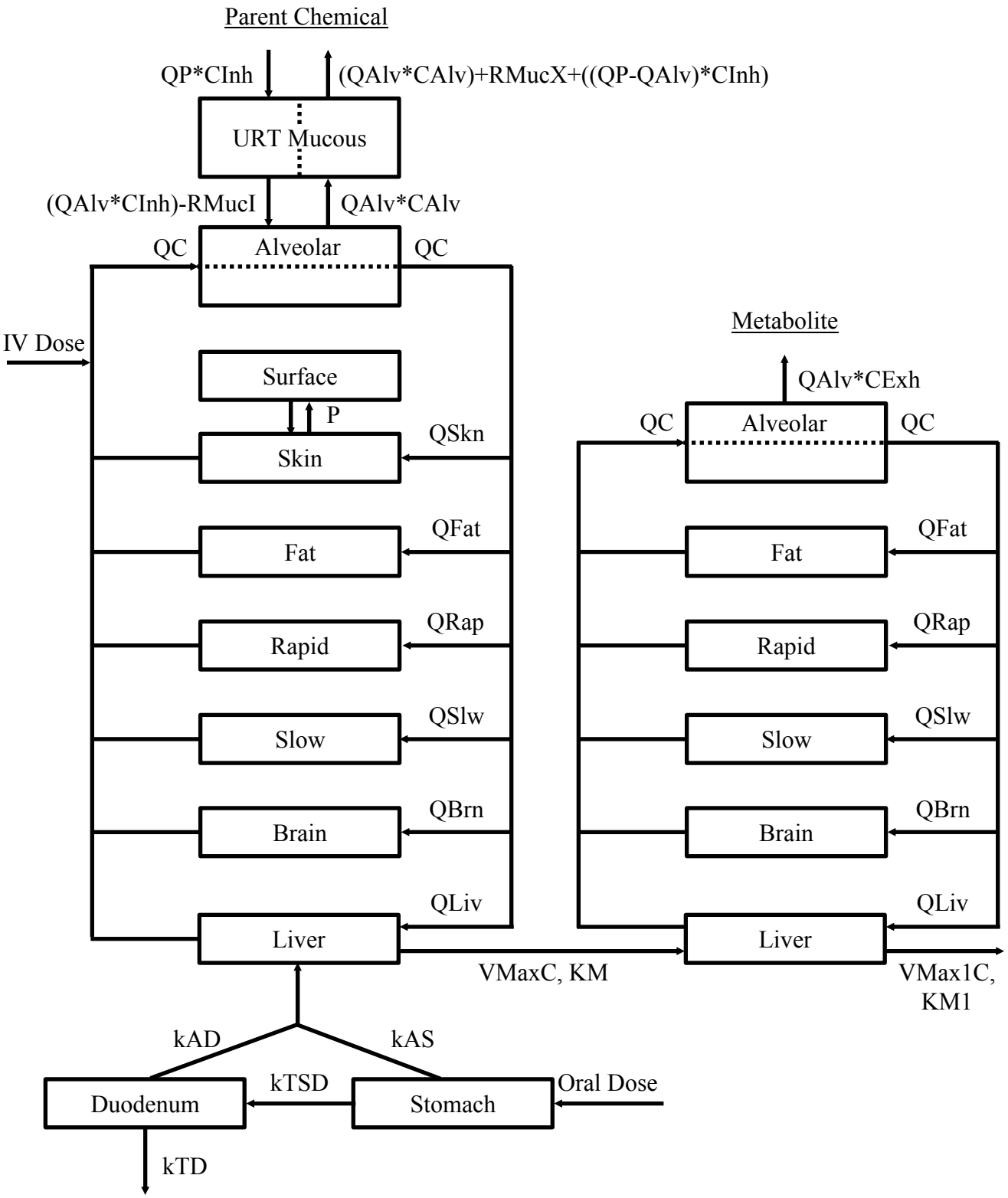

Figure 1. PB-PK Model for Isopropanol and Acetone. Abbreviations are defined in Clewell *et al*. (2001).

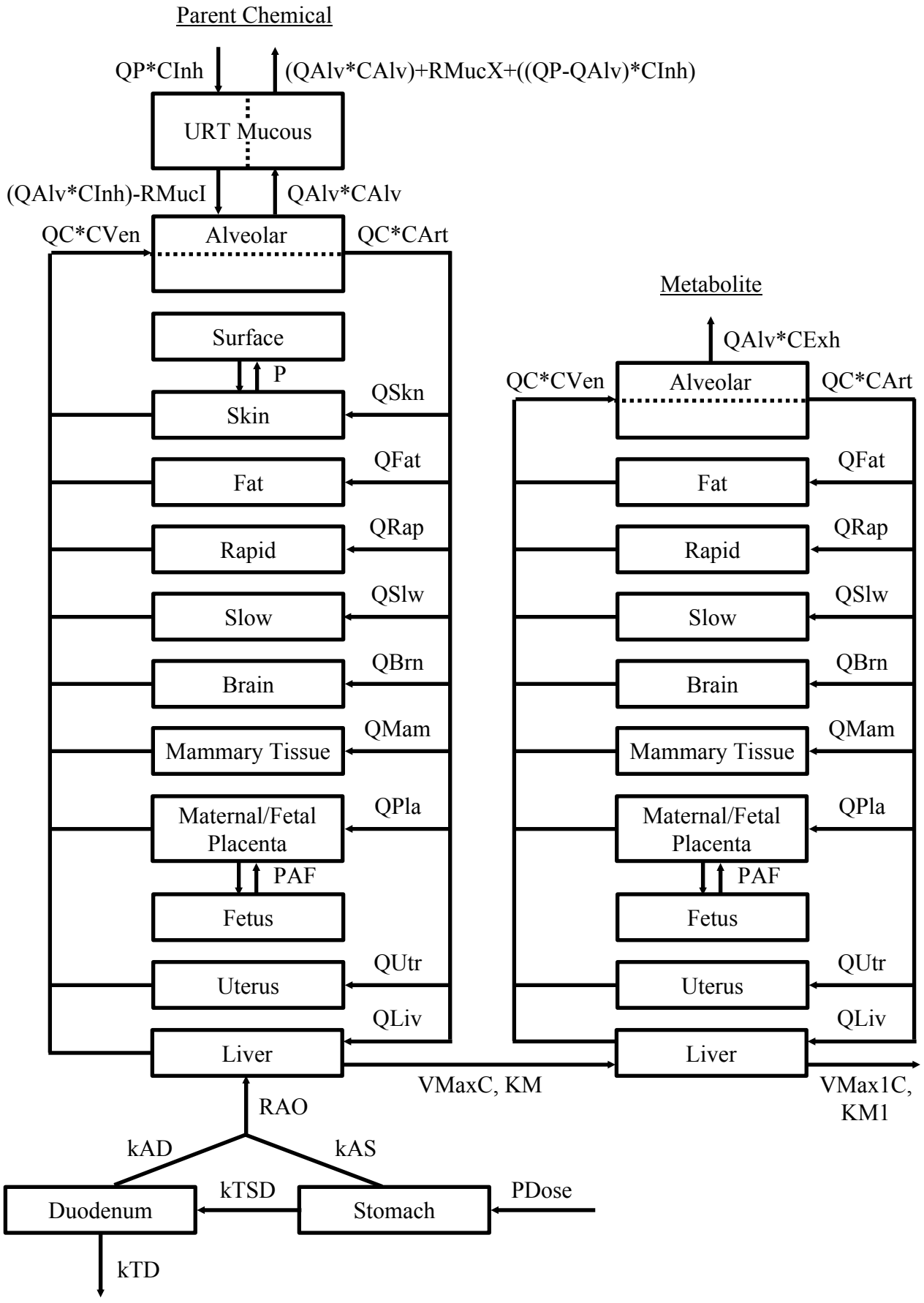

Figure 2. PB-PK Pregnancy Model for Isopropanol and Acetone. Abbreviations are defined in Gentry *et al*. (2001).