6 Quantitative Risk Assessment Based on Animal Data

6.1 Introduction

6.1.1 Diacetyl

Dose-response data for diacetyl toxicity in laboratory animals are available, and there are limited but useful animal data on the toxicity of 2,3-pentanedione. Although the NIOSH REL for diacetyl is based on the analysis of human data described in Chapter 5, NIOSH has assessed the animal data for diacetyl to determine whether they are consistent with the human data. For 2,3-pentanedione, NIOSH has conducted a comparative potency analysis, comparing the toxicity of inhaled 2,3-pentanedione to that of diacetyl. These quantitative risk assessments are described below. NIOSH interpretation of the findings and implications for occupational exposure recommendations for diacetyl are described below and in Chapter 7.

Laboratory animal studies designed to evaluate the effects of exposure to butter flavoring vapor or of diacetyl alone have demonstrated a relationship between exposure and respiratory effects. In rats exposed by inhalation to butter flavoring vapor for 6 hours (diacetyl concentrations ranged from 203 to 352 ppm), rhinitis (at the lowest exposure concentration) and bronchitis (at the higher two exposure concentrations) were observed one day after exposure [Hubbs et al. 2002]. In a follow-up study rats were exposed by inhalation to diacetyl (intermittently or continuously for up to 6 hours), which resulted in various adverse respiratory effects including epithelial necrosis and inflammation in the nose, larynx, trachea, and bronchi [Hubbs et al. 2008]. The nasal region was observed to be the most sensitive. Morgan et al. [2008] reported similar adverse respiratory effects in mice exposed by inhalation to diacetyl for up to 12 weeks. Adverse nasal and lung effects were observed with the latter found in the bronchial, peribronchial, and peribronchiolar regions.

The NTP has issued findings from a 90-day inhalation study of diacetyl in both mice and rats [National Toxicology Program 2011]. Adverse effects were observed in the nose, larynx, trachea, and bronchi in mice and rats. Because the 2011 NTP study had the longest exposure durations among all experimental animal studies, included two species, and used more animals per dose group than the Morgan et al.[2008] study, it was used in the dose-response analysis to BMDs, the lower bound on the BMDs (BMDLs), and corresponding human equivalent concentrations (HECs), as discussed below.

6.1.2 2,3-Pentanedione

Histopathological data from repeated-exposure inhalation toxicology studies with 2,3-pentanedione were first published in 2012, but are limited to 2-week exposures using small numbers of animals [Morgan et al. 2012]. Although these data are limited, it is possible to compare the toxicity produced by 2,3-pentanedione to that produced by diacetyl under similar conditions, and thus estimate the potency of 2,3-pentanedione relative to diacetyl. Therefore,

the limited toxicological data for 2,3-pentanedione are not used directly to establish a REL for 2,3-pentanedione, but only to develop an estimate of the toxic potency of 2,3-pentanedione relative to that of diacetyl. Like diacetyl, 2,3-pentanedione is a reactive alpha-dicarbonyl compound that can damage protein [Epperly and Dekker 1989; Morgan et al. 2016]. In acute inhalation studies, 2,3-pentanedione has respiratory epithelial toxicity comparable to diacetyl [Hubbs et al. 2012]. Recently, bronchial fibrosis has been documented in rats inhaling either 2,3-pentanedione or diacetyl for 2 weeks [Morgan et al., 2016].

6.2 Methods

6.2.1 Data

6.2.1.1 Diacetyl

The response data that were analyzed were obtained from the experimental study reported by the NTP [2011]. Male and female Wistar-Han rats and male and female B6C3F$_1$ hybrid mice were exposed to diacetyl vapors at concentrations of 6.25, 12.5, 25, 60, and 100 ppm, 6 hours per day, 5 days per week, for 13 weeks. The microscopic evaluations of tissues from the larynx, lung, nose, and trachea described whether or not one or more lesions were detected, the types of lesions that were detected, and the assignment of a numeric score describing the lesion's severity on an ordinal scale (1-minimal, 2-mild, 3-moderate, 4-marked) for each type that was detected. Descriptions of the types of lesions observed among rats and mice that were considered for this analysis are given in Tables 6-1 and 6-2, respectively.

6.2.1.1 2,3-Pentanedione

The results of a 2-week inhalation study of 2,3-pentanedione toxicity were reported by Morgan et al. [2012]. Individual animal data

Table 6-1. Respiratory system lesions observed in rats exposed to diacetyl that were considered for this analysis

Tissue	Response
Larynx	Inflammation, Chronic Active
Larynx	Epithelium, Necrosis
Larynx	Respiratory Epithelium, Hyperplasia
Larynx	Respiratory Epithelium, Metaplasia, Squamous
Larynx	Respiratory Epithelium, Regeneration (Females only)
Larynx	Squamous Epithelium, Hyperplasia*
Lung	Infiltration Cellular, Histiocyte
Lung	Inflammation, Eosinophil or Acute
Lung	Bronchiole, Epithelium, Hyperplasia
Lung	Bronchus, Inflammation, Chronic (Males only)
Lung	Bronchus, Epithelium, Hyperplasia†
Lung	Bronchus, Epithelium, Necrosis
Lung	Bronchus, Epithelium, Regeneration
Nose	Inflammation, Suppurative
Nose	Lymphoid Tissue, Hyperplasia
Nose	Olfactory Epithelium, Atrophy
Nose	Olfactory Epithelium, Degeneration
Nose	Olfactory Epithelium, Metaplasia, Respiratory
Nose	Olfactory Epithelium, Necrosis
Nose	Respiratory Epithelium, Hyperplasia
Nose	Respiratory Epithelium, Metaplasia, Squamous
Nose	Respiratory Epithelium, Necrosis
Nose	Turbinate, Atrophy
Trachea	Inflammation, Chronic Active
Trachea	Epithelium, Regeneration
Trachea	Epithelium, Hyperplasia
Trachea	Epithelium, Metaplasia, Squamous
Trachea	Epithelium, Necrosis

*Includes two males classified as having mild "Squamous Epithelium, Hyperplasia, Atypical"

†Includes three males and four females classified as having mild "Bronchus, Epithelium, Hyperplasia, Atypical"

Table 6-2. Respiratory system lesions observed in mice exposed to diacetyl that were considered for this analysis

Tissue	Response	Tissue	Response
Larynx	Inflammation, Chronic Active	Nose	Olfactory Epithelium, Atrophy
Larynx	Epithelium, Necrosis	Nose	Olfactory Epithelium, Metaplasia, Respiratory
Larynx	Respiratory Epithelium, Hyperplasia	Nose	Respiratory Epithelium, Metaplasia, Squamous
Larynx	Respiratory Epithelium, Metaplasia, Squamous*	Nose	Respiratory Epithelium, Necrosis
Larynx	Respiratory Epithelium, Regeneration	Nose	Respiratory Epithelium, Regeneration¶
Larynx	Squamous Epithelium, Hyperplasia†	Nose	Turbinate, Atrophy
Lung	Bronchus, Inflammation, Chronic	Trachea	Inflammation, Chronic Active
Lung	Bronchus, Epithelium, Hyperplasia‡	Trachea	Epithelium, Degeneration or Regeneration**
Lung	Bronchus, Epithelium, Regeneration§	Trachea	Epithelium, Hyperplasia
Nose	Inflammation, Suppurative	Trachea	Epithelium, Metaplasia, Atypical Squamous

*Includes lesions classified as "Respiratory Epithelium, Metaplasia, Atypical Squamous"

†Includes lesions classified as "Squamous Epithelium, Hyperplasia, Atypical"

‡Includes lesions classified as "Bronchus, Epithelium, Hyperplasia, Atypical"

§One male classified as having a minimal "Bronchus, Epithelium, Degeneration" lesion was pooled with 10 other males having a regenerative response.

¶One male and two females classified as having a "Respiratory Epithelium, Degeneration" lesion were pooled with 20 other males, and 20 other females having the regenerative response.

**Seven males and seven females had only the regenerative response, and 12 males and 11 females had only the degenerative response.

from this study were graciously provided for this analysis by Dr. Daniel Morgan, National Institute for Environmental Health and Safety (NIEHS) (personal communication to Dr. Lauralynn Taylor McKernan, NIOSH, November 30, 2010). These data describe the pathological responses of male and female Wistar-Han rats and B6C3F1 mice exposed to 2,3-pentanedione by inhalation for 6 hours per day, 5 days per week, for 2 weeks plus 2 days. The exposure concentrations were 0 ppm, 50 ppm, 100 ppm, and 200 ppm, with six animals per dose group; nasal, tracheal, and pulmonary endpoints were assessed. The tissue and pathological endpoints that could be modeled successfully for both 2,3-pentanedione and diacetyl (for comparative purposes) are listed in Table 6-3.

In addition to the 13-week NTP bioassay data described above for diacetyl, the 2,3-pentanedione data were also compared to data for diacetyl from Morgan et al. [2008]. These data describe the pathological responses of male C57Bl/6 mice exposed to diacetyl by inhalation for 6 hours per day, 5 days per week, for either 6 or 12 weeks. The exposure concentrations were 0 ppm, 25 ppm, 50 ppm, and 100 ppm, with five animals per dose group. Nasal, tracheal, and pulmonary endpoints similar to those examined in the 2,3-pentanedione study were assessed. In addition to the data in the Morgan et al. [2008] publication, tables of individual

Table 6-3. Pathological endpoints associated with exposure to 2,3-pentanedione that were modeled in this analysis

Tissue	Description of response
Lung	Bronchus, Inflammation, Chronic
Lung	Bronchus, Epithelium, Regeneration
Nose	Inflammation, Suppurative
Nose	Olfactory Epithelium, Atrophy
Nose	Respiratory Epithelium, Metaplasia
Nose	Respiratory Epithelium, Necrosis
Nose	Respiratory Epithelium, Regeneration

animal's responses were provided by Dr. Daniel Morgan, NIEHS (personal communication to Dr. Christine Sofge, NIOSH, November 18, 2008, and November 20, 2008).

6.2.2 Analytical approach

An empirical approach based on parametric regression modeling of the ordinal response data was adopted to maximize the information available for analysis from the limited numbers of rodents* in order to assess the potency of diacetyl to increase risk and to assess the relative potency of the two chemicals.

6.2.2.1 Benchmark concentration analysis for rats exposed to diacetyl

The assessment of the potency of diacetyl to increase risk employed the benchmark dose approach that was originally proposed for risk assessment of non-cancer responses by Crump [1984]. It provides a general framework that accommodates a range of responses including responses observed on dichotomous†, ordinal, and continuous scales. It has received extensive development over the past three decades, and it has become an accepted approach for risk assessment [EPA 2012]. Benchmark concentration (BMC) estimates for the pathological endpoints listed in Table 6-1 (for rats) were based on modeling of the exposure concentrations and the associated pathology. In order to avoid the loss of information inherent in dichotomizing ordinal response data, a categorical regression procedure for ordinal data was used to estimate benchmark concentrations. Categorical regression has been previously used in the analysis of toxicological data with multiple levels of severity [Guth et al. 1997; Haber et al. 2001]. The severity scores‡ for each tissue and type of lesion were assumed to be samples from a multinomial distribution following a complementary§ cumulative logistic model fitted separately for each species and sex as follows:

$$logit(\Pr(Y_{ci} \geq j)) =$$

$$\log\left(\frac{Pr(Y_{ci} \geq j)}{1 - Pr(Y_{ci} \geq j)}\right) = \alpha_j + \beta \cdot conc_{ci}$$

where

Y_{ci} denotes the corresponding severity score of the i^{th} rodent exposed to concentration, $conc_c$,

$j \in$ element of {*observed severity scores excluding zero*} for the corresponding tissue and type of lesion,

$\Pr(Y_{ci} \geq j)$ denotes the expected proportion of response score Y_{ci} greater than or equal to j, each α_j is an unknown real-valued parameter with $\alpha_{j'} < \alpha_j$ for $j' > j$, and β is an unknown real-valued parameter describing the slope of the effect of concentration on the logit scale.

*$5 \leq n \leq 10$ rodents were used per species-sex-exposure group.

†Dichotomous responses are often referred to as quantal responses.

‡When no evidence of the lesion being modeled was detected a severity score of zero (0) was assigned.

§The term complementary discerns this model from an equivalent cumulative logistic model of $Pr(Y_{ci} \geq j)$.

The logistic model is based on the logit transformation above which maps the range of expected response proportions, $0<p<1$, to $(-\infty, \infty)$; hence, models defined in terms of the transform constrain the expected proportions to the appropriate range. It is readily parameterized so that this form of the systematic relation applies under varying conditions that are consistent with biological considerations including the redefinition of the response categories by merging them [McCullagh 1980]; this specifically includes merging them to form the dichotomous responses more familiar to toxicology while preserving the *interpretations* of the model parameters thereby facilitating its application. The method of maximum likelihood was applied in order to fit[¶] the model, and a likelihood ratio (*LR*) test for a (non-null) dose-response was performed. Adequacy of the fit was assessed by performing two statistical tests, i.e., a score test for separate slopes (a slope for each unique value of *j*) and a *LR* test for an unrestricted multinomial distribution. The null distribution of the statistic of each test was approximated by its asymptotic chi-square distribution. For those models having a significant dose-response ($P<0.05$) and an adequate fit ($P>0.05$) on both tests, BMCs were estimated corresponding to the concentrations that increased expected proportions by 0.10 over controls[**] for severity scores of 1+ (lesion was at least minimal) and 2+ (lesion exceeded minimal severity). Ninety-five percent confidence intervals for the BMC were calculated from percentiles of 200,000 samples of the asymptotic multivariate normal distribution of the MLE of the model parameters[††]; both a two-sided 95% confidence interval and a lower one-sided 95% confidence limit (BMCL) were estimated.

[¶]The Logistic procedure of SAS™ 9.3 was used.

[**](i.e., a benchmark response of 0.10 for "added risk")

[††]The function, rmvnorm, of Splus with mean=MLE and covariance matrix=estimate of Cov(MLE) was used.

6.2.2.2 Benchmark concentration analysis for mice exposed to diacetyl

Benchmark concentration estimates for the pathological endpoints listed in Table 6-2 (for mice) were developed as described above for the rat data; however, an analysis of the residual errors of the fitted models provided substantial evidence against the model for the data on mice (Figure 6-1).

These residuals have mean equal to zero asymptotically if the linear-in-concentration model is correct. However, the distribution of the residuals of Figure 6-1 is shifted above zero at 50 ppm corresponding to underprediction and the distribution is shifted below zero corresponding to overprediction at 100 ppm. Figure 6-1 provides support for making a modification of the dose-response model in a manner that allows for a reduction of the rate of increase of the response at high doses. Because mice are able to substantially alter their breathing rates in a dose-dependent manner when exposed [Larsen et al. 2009; Morgan et al. 2008] the model of the data for mice was modified to include a quadratic dose term to allow it to more closely fit the data in the high-dose region of the dose-response relationship; this term was parameterized to represent a directly proportional relationship of the change in breathing rate with concentration relative to the breathing rate of the controls. The resulting estimate for male mice exposed to diacetyl was compared with corresponding ventilation measurements provided by Dr. Daniel Morgan, NIEHS (personal communication to Randall Smith, NIOSH, June 5, 2014). In addition, two parameters allowing for adjustment of the intercepts of each sex and a third parameter allowing for adjustment of the effect of exposure were added to the model to account for the varying durations of these studies. This model was further

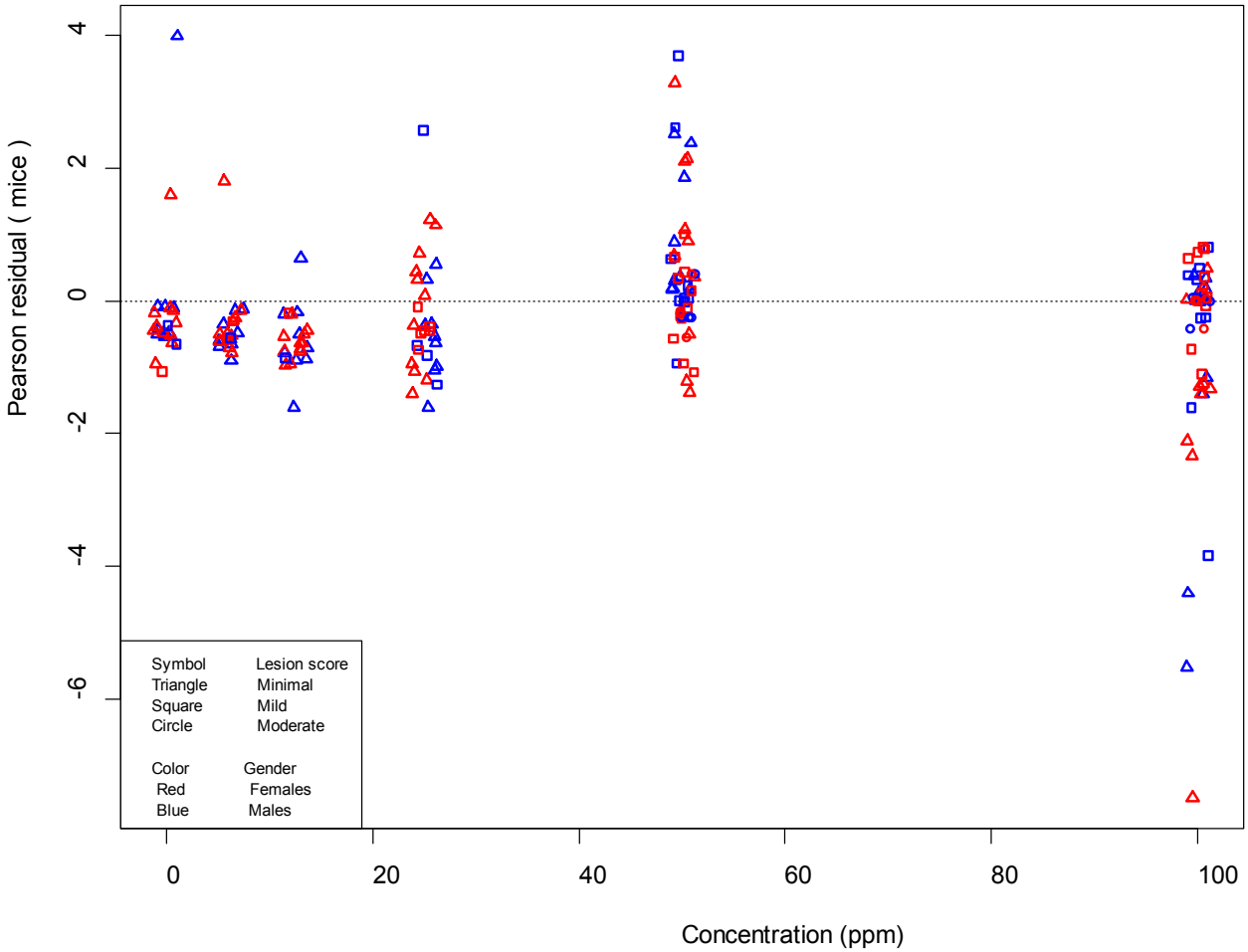

Figure 6-1. Pearson residuals of complementary cumulative logistic models with linear effect of concentration fitted to data on mice. The points have been slightly jittered horizontally to improve resolution.

extended to incorporate the comparative potency analysis of 2,3-pentanedione relative to diacetyl and incorporated an allowance for the responses of each mouse to be correlated by including random effects. It is described below in section 6.2.2.7.

6.2.2.3 Extrapolation of rodent benchmark concentrations to humans

Extrapolation of rodent BMCs to humans was based on a PBPK/CFD model for diacetyl [Gloede et al. 2011; Morris and Hubbs 2009]. The Gloede et al. [2011] extension of the Morris and Hubbs [2009] model predicts tissue concentrations of diacetyl for mucosal surfaces in the nose, trachea, bronchi, and bronchioles of rats and humans exposed to 1 ppm diacetyl. Nose-breathing and mouth-breathing humans are considered, as well as the effects of light exercise as might be expected to occur in the workplace. The Gloede et al. [2011] model assumes mouth breathing during light

exercise conditions. For extrapolation purposes, an 8-hour work day was considered to consist of 2.5 hours of sedentary exposure and 5.5 hours of light exercise, as described by the International Commission on Radiological Protection (ICRP) human respiratory tract model [ICRP 1994]. The ICRP model assumes 20 breaths per minute and a tidal volume of 1,250 mL for light exercise and 12 breaths per minute and a tidal volume of 625 mL for sedentary sitting, for a total inhalation volume of 9.6 m^3 in an 8-hour work day. Therefore, to extrapolate from rodents to humans, the BMC estimates described above were adjusted by a weighted average of the rat:human ratios of the predicted tissue concentrations for a particular anatomical region, under sedentary and light exercise conditions. The Gloede et al. [2011] estimates incorporating tissue metabolism (V_{max} for the rat, and K_{cat} for humans) were used, because local metabolism is predicted to impact significantly on the local tissue concentration [Gloede et al. 2011] (Table 3). For example, the predicted tissue diacetyl concentration for the proximal tracheal mucosa of a rat exposed to 1 ppm diacetyl is 0.33 μM, while the predicted tissue concentration for the same anatomical region is 1.4 μM in a sedentary nose-breathing human and 2.5 μM in a mouth-breathing exercising human. The rat BMCs based on pathological changes to this anatomical region were divided by a factor of (1.4 μM * 2.5 hours + 2.5 μM * 5.5 hours)/(0.33 μM * 6 hours), or 8.71. The factor of 6 hours in the denominator adjusts for the 6-hour/day duration of the experimental exposures, as compared to the 8-hour workday assumed for occupational exposures. Gloede et al. [2011] did not report tissue concentration estimates for the larynx; BMC extrapolation for this region was based on the tissue concentrations estimated for the proximal trachea. Gloede et al. [2011] reported tissue concentrations for both mainstem and small bronchi, and BMC extrapolation for bronchial endpoints were based on the mean of the rat:human ratios of tissue concentrations for mainstem bronchi and small bronchi. Rat to human scaling for the alveoli was based on the estimated fractional penetration of diacetyl through the bronchioles in the Gloede et al. PBPK model, provided by Dr. John Morris, University of Connecticut (personal communication to Dr. David A. Dankovic, NIOSH, November 8, 2012). The rat:human extrapolation factors used are shown in Table 6-4.

6.2.2.4 Extrapolation of BMCs and BMCLs from the mouse to the rat

Because no PBPK model for diacetyl exposures in the mouse is currently available, the rat PBPK model [Gloede et al. 2011] was extended to the mouse using the EPA reference concentration (RfC) methodology [EPA 1994]. In the RfC methodology, the deposition and uptake of volatile chemicals are estimated from a combination of chemical characteristics (i.e., reactivity and solubility) and the physiological characteristics of the relevant species (i.e., minute ventilation and the surface area of the relevant portion of the respiratory tract). Diacetyl is classified as a "category 1" gas in the RfC methodology because of its high water solubility. Category 1 gases are not expected to reach the pulmonary region in high concentration, but rather to be deposited primarily in the upper respiratory tract and the tracheobronchial region. This is consistent with the behavior of diacetyl in the Gloede et al. [Gloede et al. 2011] PBPK model, so that the classification of diacetyl as a category 1 gas appears to be appropriate.

Interspecies dosimetric adjustments via the RfC methodology are based on an estimate of the regional gas dose ratio (RGDR). The RGDR estimates the ratio of gas deposition with a given respiratory tract region in the two species being compared.

Table 6-4. Factors for rodent-to-human extrapolation of airway tissue concentrations of diacetyl, based on Gloede et al. [2011]

Species	Human			Human (light work)	Human (light work)
Breathing via rest/exercise	nose rest	mouth rest	mouth exercise	nose + mouth rest + exercise*	nose + mouth rest + exercise*
	Human-to-rat ratio†				Human-to-mouse ratio‡
Proximal nose	1.6	0	0	0.67	0.3
Proximal trachea	4.2	6.1	7.6	8.7	2.7
Mainstem bronchi	10.0	14.0	21.0	23.0	7.3
Small bronchi	7.2	10.0	32.2	32.0	10.0
Average bronchi§	8.6	12.0	26.6	28.0	8.7
Bronchioles	5.0	7.3	40.9	40.0	12.0
Alveoli¶	4.69	—	15.0	15.7	4.9

"Light work" was estimated to be a combination of 2.5 hours at rest, with nasal breathing, plus 5.5 hours of exercise, with mouth breathing, per 8-hour work day; this was compared to a 6-hour/day exposure for rodents in the experimental studies.

†Rat-to-human scaling based on the overall catalytic rate, K_{cat}, in Gloede et al. [2011]Table 3, except as noted below for alveoli.

‡Mouse-to-human scaling assuming mouse is 2.4 times as sensitive as the rat for nasal effects and 3.2 times as sensitive for tracheobronchial effects, based on the regional gas dose ratio (see section 6.2.2.4)

§"Average bronchi" = arithmetic mean of values for mainstem and small bronchi

¶Rat to human scaling for the alveoli was based on the estimated fractional penetration of diacetyl through the bronchioles in the Gloede et al. PBPK model.

For the ET region, the RGDR is calculated [EPA 1994], eqn. 4-18, as:

$$RGDR_{ET} = \frac{Dose_{ET_A}}{Dose_{ET_B}} \approx \frac{\left(\frac{V_E}{SA_{ET}}\right)_A}{\left(\frac{V_E}{SA_{ET}}\right)_B}$$

where:

V_E = minute volume (mL/min = cm^3/min)
SA = surface area (cm^2)
ET = a subscript denoting the extrathoracic region
A, B = subscripts denoting experimental animal and target species, respectively

For the TB region, the RGDR is calculated [EPA 1994], eqn. 4-22, as:

$$RGDR_{TB} = \frac{Dose_{TB_A}}{Dose_{TB_B}} = \frac{\left(\frac{V_E}{SA_{TB}}\right)_A}{\left(\frac{V_E}{SA_{TB}}\right)_B} \cdot \frac{\left(e^{-\left(\frac{SA_{ET}}{V_E}\right)}\right)_A}{\left(e^{-\left(\frac{SA_{ET}}{V_E}\right)}\right)_B}$$

where:

V_E = minute volume (mL/min = cm^3/min)
SA = surface area (cm^2)
TB = a subscript denoting the tracheobronchial region
ET = a subscript denoting the extrathoracic region
A, B = subscripts denoting experimental animal and target species, respectively

The values assumed for V_E and SA, and the resulting RGDR values for mouse-to-rat extrapolation, are shown in Table 6-5. The rat V_E value is based on data from Gloede et al. [2011], and the mouse V_E was taken from Morgan et al. [2008]. The SA values are from EPA [1994].

The RGDR is used to adjust a point of departure (POD), i.e., a BMC or BMCL in the laboratory species to an equivalent concentration in the target species as follows:

$POD_{BEC} = POD_A * RGDR$

where:

POD_{BEC} = POD equivalent concentration in the target species;

POD_A = POD in the experimental species; and

RGDR = Species A-to-species B regional gas dose ratio for the appropriate region of the respiratory tract.

Although the RGDR is typically used to develop human equivalent concentrations from experimental animal data, in this case it is used to develop a rat equivalent concentration for a point of departure estimated from experimental data in the mouse. The Gloede et al. [2011] PBPK model is then used to extrapolate from the rat equivalent concentration to a human equivalent concentration.

6.2.2.5 Duration adjustment and final human equivalent concentration conversions

Adjustment for the daily duration of exposure (6 hours/day for the NTP experimental study vs. 8 hours/day assumed for occupational exposures) is included in the PBPK model-based extrapolation from rodents to humans, as described in section 6.2.2.2 above; therefore, no additional adjustment for exposure hours per day is needed. The experimental exposure protocol of five exposures per week matches the assumed occupational exposure pattern, so that no adjustment for days exposed per week is required in extrapolating from animals to humans. Occupational exposures may take place for an entire working lifetime, which is assumed to be up to 45 years in duration. Ideally, the datasets used for quantitative risk assessment of occupational exposures to toxicants would include data from 2-year rodent bioassays; however, in this case the available data are limited to exposures of 13 weeks or less. An 8-fold dosimetric adjustment (104 weeks/13 weeks) could be considered in order to account for this discrepancy; however, this appears to be unnecessary for diacetyl.

Table 6-5. Calculation of RGDR for mouse-to-rat extrapolation

Species	V_E* (mL/min)	URT SA† (cm^2)	TB SA‡ (cm^2)	URT RGDR§	TB RGDR¶
Rat	264.0	15	22.5	—	—
Mouse	128.5	3	3.5	2.4	3.2

*Minute volume ventilation

†Upper respiratory tract surface area

‡Tracheobronchial surface area

§Mouse-to-rat regional gas dose ratio for the upper respiratory tract

¶Mouse-to-rat regional gas dose ratio for the tracheobronchial region

This conclusion is based on the analysis of Allen [2009a], who concluded that the 6- and 12-week mouse experiments had response rates that could be modeled together (i.e., the duration of the experiment could be ignored) for all the lesions analyzed; there did not appear to be a progression toward higher rates of response or more severe responses when the exposure level remained the same but the duration of exposure was increased from 6 to 12 weeks. However, because of the small number of animals used in this study, the power to detect differences between the 6-week and 12-week experiments is limited. As a consequence of the limited duration of the experimental studies and the limited ability to detect differences between the responses at 6 and 12 weeks, the possibility of increased toxicity with lifetime exposure cannot be entirely ruled out. This possibility was addressed through the application of an uncertainty factor (UF) – discussed below – rather than a dosimetric adjustment.

6.2.2.6 Application of uncertainty factors

The HECs are estimates of frankly toxic exposure levels, and must be adjusted by the application of UFs to allow for uncertainty in animal-to-human extrapolation, interindividual variability, and less than lifetime exposure. In general, these UFs are assumed to be 10-fold for animal-to-human extrapolation and another 10-fold for interindividual variability. The animal-to-human extrapolation can be subdivided into a factor of 4 for pharmacokinetics and a factor of 2.5 for interspecies variability in susceptibility [WHO 1994]. In this case, the interspecies pharmacokinetic factor is replaced by the use of the Gloede et al. [2011] pharmacokinetic model, leaving an interspecies UF of 2.5. The UF for interindividual variability can be subdivided into two factors of $\sqrt{10}$, or 3.2, one for interindividual variability in pharmacokinetics and the other for interindividual variability in susceptibility [WHO 1994]. Because the toxicity of diacetyl occurs at the point of contact with respiratory tract mucosa there is relatively little opportunity for interindividual variability in pharmacokinetics, and so the first subfactor is not applied. However, interindividual variability in susceptibility to toxicity cannot be ruled out; therefore, a factor of 3.2 is applied. In addition, a factor of 3 is applied for conversion from subchronic to chronic exposure. When the three factors (3.2-fold for interindividual variability, 2.5-fold for interspecies variability, and 3-fold for subchronic to chronic) are multiplied, the resulting total UF is 24.

6.2.2.7 Joint analysis of the data on mice from the diacetyl and 2,3-pentanedione bioassays

To avoid the loss of information inherent in dichotomizing ordinal data the severity scores of each type of lesion observed among nasal and lung tissues were modeled as having been sampled from conditional multinomial distributions given the unobserved random effects associated with each mouse described by the following family of complementary cumulative logistic models:

$$logit\left(Pr\left(Y_{bskcr(t)i} \geq j\right)\right) = log\left(\frac{Pr\left(Y_{bskcr(t)i} \geq j\right)}{1-Pr\left(Y_{bskcr(t)i} \geq j\right)}\right)$$
$$= \alpha_{sjr(t)} + u_{bskci} + \omega_s \cdot \tau_{bskci}$$
$$+ f_{bskcti}\beta_{sjr(t)}\{m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{skt}, \gamma_s)\} \cdot conc_{bskci},$$

where

$\alpha_{sjr(t)} + u_{skci} + \omega_s \cdot \tau_{bskci}$ describes effects in the absence of exposure,

$f_{bskcti}\beta_{sjr(t)}\{m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{skt}, \gamma_s)\} \cdot conc_{bskci}$ describes effects of exposure

and

b indexes the bioassay study

s indexes sex,

$k = 0 \leftrightarrow$ 2,3-pentanedione exposure and $k = 1 \leftrightarrow$ diacetyl exposure,

bkc identifies the exposure group and $conc_{bkc}$ is the corresponding exposure concentration,

$i = 1, \ldots, n_{bskc}$ indicates each of the mice within the exposure group identified by $bskc$ and $conc_{bskci}$ denotes the corresponding exposure concentration,

$r(t)$ identifies the response lesion, r nested within tissue, t, (lung or nasal),

$Y_{bskcr(t)i}$ is the response variable that is integer-valued based on the assigned severity score and it ranges over {0,1,2,3} for all response lesions[‡‡] except necrosis of the respiratory epithelium of the nose where the range was {0,1,2},

$Pr(Y_{bskcir(t)} \geq j)$ represents the expected proportion having response severity score greater than or equal to j for $j \in \{1, \ldots, \max(Y_{bskcr(t)i})\}$,

$\alpha_{sjt(r)}$ denotes the intercept parameters for lesion $r(t)$ which are subject to constraints

$\alpha_{s2t(r)} - \alpha_{s1t(r)} = \Delta\alpha_{s2} < 0$ and

$\alpha_{s3t(r)} - \alpha_{s2t(r)} = \Delta\alpha_{s3} < 0$ thus ensuring[§§] $\alpha_{s3t(r)} < \alpha_{s2t(r)} < \alpha_{s1t(r)}$,

$u_{bskci} \sim N(0, \sigma^2_{su})$ is a normally distributed random effect associated with the i^{th} mouse of $bskc$; likelihood ratio tests of null values of the variance parameters, σ^2_{us}, were performed[¶¶] and subject to being incorporated into the model.

$\omega_s \cdot \tau_{bskci}$ represents an adjustment to the intercepts allowing for effects associated with the longer durations quantified by τ_{bskci} of the diacetyl studies described by the unknown parameter, ω_s,

$\beta_{sjr(t)}$ are slope parameters for the effect exposure to 2,3-pentanedione, which are subject to constraints $\beta_{s2t(r)} - \beta_{s1t(r)} = \Delta\beta_{s2} \leq 0$ and $\beta_{s3t(r)} - \beta_{s2t(r)} = \Delta\beta_{s3} \leq 0$ thus ensuring[***] $\beta_{s3r(t)} \leq \beta_{s2r(t)} \leq \beta_{s1r(t)}$.

A test of $\Delta\beta_{s2} = \Delta\beta_{s3} = 0$ was performed[‡‡] and subjected to incorporation.

The slope parameters are subject to modification by the multiplicative function,

$$m(s, k, conc_{bskci}, t, \tau_{bskci}; \emptyset_{sr(t)}, \varphi_{skt}, \gamma_s) = [1+\gamma_s \cdot \tau_{bskci}][1+I(k=1)\cdot(\emptyset_{sr(t)}-1) + \varphi_{skt} \cdot conc_{bskci}]$$

where the factor,

$[1+\gamma_s \cdot \tau_{bskci}]$, describes an adjustment for the longer durations of the diacetyl studies parameterized by $\gamma_s > -1/max(\tau_{bskci})$; however, the assumption, $\gamma_s = \gamma$, was imposed because information was absent from female mice on this parameter, the diacetyl indicator, $I(k=1)=1$, when $k=1$ and $I(k=1)=0$ when k=0, $\emptyset_{sr(t)}$ are parameters describing the potency of diacetyl relative to 2,3-pentanedione

[‡‡]When no evidence of the lesion being modeled was detected a severity score of zero (0) was assigned.

[§§]Hence, the requirement that $r(Y_{kcit(r)} \geq 3) < Pr(Y_{kcit(r)} \geq 2) < Pr(Y_{kcit(r)} \geq 1)$ is satisfied for the controls.

[¶¶]Whenever the fitted values of the parameters were null, i.e., 0, the test statistic $-2\log(Likelihood\ ratio) = 0$ and the test was deemed to be nonsignificant.

[***]Hence, the requirement that $Pr(Y_{kcit(r)} \geq 3) < Pr(Y_{kcit(r)} \geq 2) < Pr(Y_{kcit(r)} \geq 1)$ is satisfied globally.

at low doses for {r(t)}; the hypothesis, $\emptyset_{sr(t)} = \emptyset_s$, was tested and subject to being incorporated into the model, and

φ_{skt} allows for an adjustment for a quadratic effect of concentration that may be attributed to directly proportional changes in respiratory ventilation with concentration where φ_{skt} is the constant of proportionality in units of controls' ventilation; thus φ_{skt} describes the change relative to controls. The hypothesis, $\varphi_{sk,lung} = \varphi_{sk,nose} = \varphi_{sk}$, was tested and subject to being incorporated into the model.

f_{bskcti} is one of a pair of lognormally distributed random effects (one effect per tissue indicated by t) of the i^{th} mouse of exposure group *bskc* acting multiplicatively on the effect of dose. Thus, an allowance for multiplicative variations from mouse to mouse by tissue-specific positive factors acting on the magnitudes of the slope parameters was incorporated. Each f_{bskcti} was modeled as having unit expectation and variance $(e^{\sigma_{st}^2}-1)$; thus, the variance of log $(f_{bskcti}) = \sigma_{st}^2$, $t = 1, 2$ for the *lung* and *nose*, respectively, together with an associated covariance parameter σ_{s12}. The hypothesis that lognormal random effects are independent was examined by testing $\sigma_{s12} = 0$ and was subject to being incorporated. Furthermore, the hypothesis that only one lognormal random effect for each mouse was necessary, i.e., $f_{bskc1i} \equiv f_{bskc2i}$ was tested and subject to being incorporated.

Model development proceeded by sequentially fitting a series of nested models of increasing complexity with all random effects omitted. This was advantageous for obtaining initial estimates of the fixed effects parameters for fitting a corresponding model that included random effects. Models were fitted by the method of maximum likelihood; the likelihoods of models containing unobserved random effects were obtained by integrating out these effects using adaptive Gaussian quadrature as described by Pinheiro and Bates [1995]. Likelihood ratio tests were performed to test hypotheses about model parameters and associated P values were based on the chi-square approximation to $-2\log (LR)$. Evidence against incorporating the previously described restrictions on model parameters was deemed significant if the P value of the corresponding test was less than 0.05 for selecting the model on which to base the estimation of relative potency parameters and benchmark concentrations.

The model selected for estimation of relative potencies and BMCs contained three lognormal random effects parameters and 53 fixed-effects parameters; it had the following form:

$$logit\left(Pr\left(Y_{bskcr(t)i} \geq j\right)\right) = log\left(\frac{Pr\left(Y_{bskcr(t)i} \geq j\right)}{1-Pr\left(Y_{bskcr(t)i} \geq j\right)}\right)$$

$$= \alpha_{sjr(t)} + \omega_s \cdot \tau_{bskci} + f_{bskcti}\beta_{sr(t)}\{m(s,\ k,\ conc_{bskci},\ t,\ \tau_{bskci};\ \theta_{sr(t)},\ \varphi_{sk},\ \gamma)\} \cdot conc_{bskci}$$

where $m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{sk}, \gamma)$

$$= [1+\gamma \cdot \tau_{skci}]\ [1+I(k=1)\cdot(\theta_{sr(t)}-1)\ +\varphi_{sk} \cdot conc_{kci}]$$

i.e., this model was simplified by incorporating the following:

Null values of the variance parameters, σ_{ius}^2 [intercept random effects omitted],

$\Delta\beta_{s3} = \Delta\beta_{s2} = 0 \rightarrow \beta_{s3r(t)} = \beta_{s2r(t)} = \beta_{s1r(t)} = \beta_{sr(t)}$ [single 2,3-pentanedione slope parameter for each $sr(t)$],

Separate relative potency parameters, $\emptyset_{sr(t)}$ were retained since significant evidence against the hypothesis $\emptyset_{sr(t)} = \emptyset_s$ was obtained; hence, $\emptyset_{sr(t)}\beta_{sr(t)}$ describes the corresponding diacetyl slope for each *sr(t)*,

$\varphi_{sk,lung} = \varphi_{sk,nose} = \varphi_{sk}$ [quadratic effect independent of tissue],

$MLE(\sigma_{st}^2) = 0$ for lognormal random effects of nasal responses of female mice was replaced by nullifying this parameter,

The adequacy of a single lognormal random effect was rejected,

Independence of the lognormal random effects for lung and nasal tissues of male mice [implied by acceptance of $\sigma_{s12} = 0$] was assumed.

The model was coded and fitted using the NLMixed procedure of SAS™ 9.3. At least two lines of evidence provided support that the algorithm for fitting the model converged to a solution for the parameters that was a unique optimum as follows: (1) The Hessian matrix of the fit was positive definite††† and (2) exploration of the likelihood surface in a neighborhood of the solution via examination of likelihood profiles supported its optimality in all cases that were examined. Hence, this evidence supports the identifiability of the parameters of the model with these data suggesting that the model is not overparameterized.

The fit of the model was assessed by calculating grouped‡‡‡ Pearson residuals conditional on the empirical Bayes estimates of the random effects $\left(\hat{\underline{f}}_{bskcti}^{(eB)}\right)$ for each tissue-response as follows:

$$r_{bskcr(t)j}^{cP} = \frac{\sum_{i\in bskcr(t)j} obs_{bskcr(t)ji} - \sum_{i\in bskcr(t)} \hat{E}\left(obs_{bskcr(t)ji} | \hat{\underline{f}}_{bskcti}^{(eB)}\right)}{\sqrt{\sum_{i\in bskcr(t)j} \widehat{Var}\left(obs_{bskcr(t)ji} | \hat{\underline{f}}_{bskcti}^{(eB)}\right)}}$$

where the fitted expectations and variances of each mouse were based on the associated binomial distribution of a factoring of the conditional multinomial likelihood into its conditionally independent binomial components corresponding to the "outcomes" $(Y \geq 1 | \underline{f})$, $(Y \geq 2 | Y \geq 1, \underline{f})$, and $(Y \geq 3 | Y \geq 2, \underline{f})$.

Furthermore, a saturated fixed-effects model with random effects omitted was compared to the selected model by examination of twice the difference of log*(Likelihood)* values relative to the difference in the number of parameters. Finally, an ad hoc procedure was applied wherein binomial deviance residuals corresponding to factoring the multinomial likelihood of the corresponding 53 parameter model (with random effects omitted) into a product of conditional binomial terms were used to estimate a factor for adjusting the width of the confidence intervals analogous to an adjustment for overdispersion because the model-based confidence intervals may be too narrow if the model is incorrect. Two-sided 95% confidence limits with and without adjustment were calculated from application of a normal approximation to the natural logarithms of the relative potencies and the BMCs associated with a 10% benchmark response for additional risk.§§§

†††NLMixed minimizes –log(L) and it provides a warning if its criteria for a positive definite Hessian is not satisfied; no such warning was given.

‡‡‡The term "grouped" is to clarify that they are based on summing the observed and fitted expectations and variances over the mice within each treatment group defined by each unique combination of *b x k x s x c*.

§§§i.e., $Pr(Y_{skcr(t)} \geq j \mid conc = BMC_{jskr(t)}, f_{skcti} = 1) - Pr(Y_{skcr(t)} \geq j \mid conc = 0, f_{skcti} = 1) = 0.10$.

6.2.2.8 Benchmark concentration analysis using quantal models

To explore the impact of the categorical regression procedure described above on the BMC estimates for diacetyl, the data for the pathological endpoints listed in Table 6-1 (for rats) and Table 6-2 (for mice) were also dichotomized, and alternative benchmark concentration estimates were developed using quantal modeling and model averaging. Any response of minimal or greater severity was treated as a positive response, and the model averaging procedure was based on fitting the multistage, Weibull, and log-probit models, as described by Wheeler and Bailer [2007]. Only datasets with two or more partial response groups were modeled. The benchmark response rate was set at 10%, and the resulting BMC and BMCL estimates are shown in Table 6-9. Only models with an average-model *P* value of 0.05 or greater were considered to fit the data adequately.

6.3 Results

6.3.1 Diacetyl

BMC and BMCL estimates based on diacetyl toxicity in rats and mice were developed as described in sections 6.2.2.1 and 6.2.2.7, respectively. Not all of the pathological endpoints listed in Tables 6-1 and 6-2 could be adequately modeled. The rat endpoints that could be modeled adequately according to the criteria listed in section 6.2.2.1 (a score test for separate slopes and a likelihood ratio test for an unrestricted multinomial distribution) are shown in Table 6-6. Mouse endpoints that could be modeled adequately by the criteria described in section 6.2.2.7 are shown in Tables 6-7 and 6-8. The associated ventilation coefficient[""] of diacetyl among males was −0.378 ± 0.0582 and among females it was −0.530 ± 0.357.

The BMC and BMCL estimates were extrapolated to HECs as described in sections 6.2.2.2 – 6.2.2.4, and the HECs were converted to candidate REL values by the application of UFs as described in section 6.2.2.5. The BMC/BMCL values for rats, and their corresponding HEC and candidate REL values are shown in Table 6-6. The BMC/BMCL values for mice, and their corresponding HEC and candidate REL values are shown in Tables 6-7 and 6-8; the BMCL values in Table 6-7 have not been adjusted for overdispersion, while the BMCL values in Table 6-8 have been adjusted for overdispersion. Scatter plots of the 359 grouped Pearson residuals calculated from the data on mice indicated they were positively skewed at low concentrations and negatively skewed at high concentrations. Hence, they were not approximately normally distributed, which is to be expected given the discrete nature of the response data and the small numbers of mice in each treatment group ($5 \leq n \leq 10$). Although evidence of systematic departures of the residuals was not apparent, 13 of the residuals indicated deviations from the fit of the joint model of diacetyl and 2,3-pentanedione by more than three standard errors (not shown). Although less than one such residual deviation would be expected for normally distributed residuals the observation of 13 such deviations seems suggestive that extraneous variations may be present and motivated our having increased the widths of model-based confidence limits by the application of an overdispersion factor of 1.61 for adjusting the model-based standard errors.

Overall, the BMCs range from 16.8–68 ppm diacetyl, and the BMCLs range from 10–49.9 ppm diacetyl. After interspecies pharmacokinetic adjustments based on the Gloede et al. [2011] model, the human-equivalent BMCL values (BMCL_HECs) range from 1.4–95.8 ppm diacetyl, and the BMCL candidate REL

[""]Estimate of $\varphi_{s,diacetyl}$ ± Model-based standard error per 100 ppm.

values (after the application of uncertainty factors) range from 0.06–4.0 ppm diacetyl.

6.3.1.1 Sensitivity analysis

As a sensitivity analysis, alternative BMC and BMCL values were also derived for the NTP [2011] diacetyl study by dichotomizing the data, fitting quantal models, and model averaging, as described in section 6.2.2.8. The model average BMCs ranged from 14.6–78 ppm, with BMCLs of 2.4–57.9 ppm. The model average BMCs and BMCLs were extrapolated to humans as described above for the categorical-regression derived BMCs/BMCLs. The $BMCL_{HEC}$ values ranged from 0.9–54.3 ppm, and the $BMCL_{REL}$ values ranged from 0.04–2.26. As shown in Table 6-9, if the candidate RELs were derived from the quantal modeling rather than categorical regression, the lowest candidate REL value would be reduced from 0.06 ppm to 0.04 ppm.

Another assumption made in this risk assessment is that toxicity observed in mice can be scaled to rats using the EPA [1994] RfC methodology to estimate a mouse-to-rate respiratory dose ratio, or RGDR. It was assumed that this extrapolation is best performed on the basis of measured values of respiratory ventilation, as opposed to estimating respiratory ventilation on the basis of body weight. As detailed above in section 6.2.2.4, use of the measured respiratory ventilation rates leads to RGDRs of 2.4 for upper-respiratory toxicity and 3.2 for lower respiratory toxicity. The impact of the decision to use measured respiratory rates in the RGDR calculation was evaluated by a comparison to the RGDRs which would be obtained using the default RfC methodology, based on body weights, and described in EPA [1994]. Using the EPA [1994] default methodology, in which the respiratory ventilation rate is estimated from the animal biody weight, results in RGDRs of 1.15 for upper respiratory tract effects and 1.5 for lower respiratory tract effects. Therefore the mouse-to-rat scaling factor would be approximately halved, and as shown in Table 6-9 the lowest candidate REL value would be reduced to 0.03 ppm, based on chronic bronchial inflammation in the female mouse lung.

A key assumption made in this risk assessment is that the Gloede et al. [2011] PBPK model is the most appropriate method for extrapolating from rats to humans. A possible alternative would be to use the EPA [1994] RfC methodology to estimate animal-to-human scaling factors, based on the RGDR. Measured respiratory ventilation values are available for mice and rats, as used in section 6.2.2.4, and the human occupational respiration rate can be assumed to be 20 L/min. Using these values and the EPA [1994] procedures for category 1 gases, the estimated RGDRs for rat-to-human extrapolation are 0.18, 1.9, and 2.1 for the upper respiratory tract, the tracheobronchial region, and the pulmonary tract, respectively. Corresponding values for mouse-to-human extrapolation are 0.43, 5.9, and 6.9 for the upper respiratory tract, the tracheobronchial region, and the pulmonary tract, respectively. These RGDRs would replace the Gloede et al. PBPK model for extrapolating from rats to humans, and would result in candidate RELs ranging from 0.15–16.1 ppm for BMCs, and from 0.10–14.3 ppm for BMCLs. The lowest candidate REL derived using the RGDR method would be 0.10 ppm, as opposed to 0.06 ppm using the Gloede et al. [2011] model. The endpoints yielding the lowest alternative candidate REL values from the sensitivity analysis are shown in Table 6-9, along with the lowest of the candidate RELs from the main analysis, for comparison.

Table 6-6. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits based on toxicity in rats exposed to diacetyl

Sex	Tissue	Response	Separate slope *P* value†	Likelihood ratio *P* value‡	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Infiltration cellular, histiocyte	0.49	0.45	43	30	15.70	2.7	1.9	24	0.11	0.08
M	Lung	Inflammation, eosinophil or acute	0.055	0.35	29	22	15.70	1.8	1.4	24	0.08	0.06
M	Nose	Olfactory epithelium, degeneration	0.59	0.95	20	13	0.66	30.3	19.7	24	1.26	0.82
M	Nose	Olfactory epithelium, metaplasia, respiratory	0.67	0.78	41	27	0.66	62.1	40.9	24	2.59	1.70
M	Nose	Olfactory epithelium, necrosis	0.23	0.62	27	19	0.66	40.9	28.8	24	1.70	1.20
M	Trachea	Epithelium, hyperplasia	0.28	0.81	68	47	8.70	7.8	5.4	24	0.33	0.23
F	Nose	Inflammation, suppurative	0.12	0.59	22	15	0.66	33.3	22.7	24	1.39	0.95
F	Nose	Lymphoid tissue, hyperplasia	0.83	0.20	23	18	0.66	34.8	27.3	24	1.45	1.14
F	Nose	Turbinate, atrophy	0.42	>0.99	36	24	0.66	54.5	36.4	24	2.27	1.52

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

†Chi-square test *P* value for separate slopes for severity scores; $P > 0.05$ considered to indicate an adequate model fit by this criterion.

‡Chi-square test *P* value for a likelihood ratio test for an unrestricted multinomial distribution; $P > 0.05$ considered to indicate an adequate model fit by this criterion.

Table 6-7. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits based on toxicity in mice exposed to diacetyl; BMCLs not adjusted for overdispersion

Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Bronchus, inflammation, chronic	41.8	27.4	8.7	4.8	3.1	24	0.20	0.13
M	Lung	Bronchus, epithelium, regeneration	54.2	38.1	8.7	6.2	4.4	24	0.26	0.18
M	Nose	Inflammation, suppurative	30.5	24.7	0.28	109.0	88.2	24	4.54	3.68
M	Nose	Olfactory epithelium, atrophy	32.3	23.0	0.28	115.5	82.1	24	4.81	3.42
M	Nose	Respiratory epithelium, metaplasia, squamous	26.5	19.2	0.28	94.8	68.6	24	3.95	2.86
M	Nose	Respiratory epithelium, necrosis	36.0	26.8	0.28	128.5	95.9	24	5.35	4.00
M	Nose	Respiratory epithelium, regeneration	40.2	23.5	0.28	143.7	83.9	24	5.99	3.50
F	Lung	Bronchus, inflammation, chronic	19.4	15.3	8.7	2.2	1.8	24	0.09	0.08
F	Lung	Bronchus, epithelium, regeneration	56.1	49.9	8.7	6.5	5.7	24	0.27	0.24
F	Nose	Inflammation, suppurative	27.0	22.9	0.28	96.5	81.7	24	4.02	3.40
F	Nose	Olfactory epithelium, atrophy	22.0	17.2	0.28	78.5	61.4	24	3.27	2.56
F	Nose	Respiratory epithelium, metaplasia, squamous	21.8	17.8	0.28	77.7	63.7	24	3.24	2.65
F	Nose	Respiratory epithelium, necrosis	16.8	12.2	0.28	59.8	43.5	24	2.49	1.81
F	Nose	Respiratory epithelium, regeneration	18.7	13.4	0.28	66.6	47.8	24	2.78	1.99

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

Table 6-8. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits based on toxicity in mice exposed to diacetyl; BMCLs adjusted for overdispersion

Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Bronchus, inflammation, chronic	41.8	21.2	8.7	4.8	2.4	24	0.20	0.10
M	Lung	Bronchus, epithelium, regeneration	54.2	30.8	8.7	6.2	3.5	24	0.26	0.15
M	Nose	Inflammation, suppurative	30.5	21.7	0.28	109.0	77.6	24	4.54	3.23
M	Nose	Olfactory epithelium, atrophy	32.3	18.7	0.28	115.5	66.7	24	4.81	2.78
M	Nose	Respiratory epithelium, metaplasia, squamous	26.5	15.8	0.28	94.8	56.3	24	3.95	2.35
M	Nose	Respiratory epithelium, necrosis	36.0	22.5	0.28	128.5	80.2	24	5.35	3.34
M	Nose	Respiratory epithelium, regeneration	40.2	16.9	0.28	143.7	60.5	24	5.99	2.52
F	Lung	Bronchus, inflammation, chronic	19.4	13.3	8.7	2.2	1.5	24	0.09	0.06
F	Lung	Bronchus, epithelium, regeneration	56.1	46.4	8.7	6.5	5.3	24	0.27	0.22
F	Nose	Inflammation, suppurative	27.0	20.7	0.28	96.5	73.9	24	4.02	3.08
F	Nose	Olfactory epithelium, atrophy	22.0	14.8	0.28	78.5	52.9	24	3.27	2.20
F	Nose	Respiratory epithelium, metaplasia, squamous	21.8	15.8	0.28	77.7	56.5	24	3.24	2.35
F	Nose	Respiratory epithelium, necrosis	16.8	10.0	0.28	59.8	35.9	24	2.49	1.50
F	Nose	Respiratory epithelium, regeneration	18.7	10.9	0.28	66.6	39.0	24	2.78	1.63

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

Table 6-9. Alternate benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits developed as a sensitivity analysis for key risk assessment assumptions

Species	Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
Rat	Male	Lung	Eosinophilic inflammation*	29	22	15.7	1.8	1.4	24	0.08	0.06
Mouse	Female	Lung	Bronchus, inflammation, chronic†	19.4	13.3	8.7	2.2	1.5	24	0.09	0.06
Mouse	Female	Larynx	Chronic inflammation‡	16	2.4	2.7	5.9	0.9	24	0.25	0.04
Mouse	Male	Lung	Bronchus, inflammation, chronic§	19.4	13.3	18.7	1.0	0.7	24	0.04	0.03
Rat	Male	Nose	Olfactory epithelium, degeneration¶	20	13	0.18**	3.6	2.3	24	0.15	0.10

*From main analysis, Table 6-6, for comparison to alternate estimates.

†From main analysis, Table 6-8, for comparison to alternate estimates.

‡Alternative analysis based on quantal modeling.

§Alternative analysis using respiratory ventilation rates based on body weight, rather than measured values.

¶Alternative analysis using EPA [1994] RfC methodology rather than the Gloede et al. [2011] PBPK model.

**The animal-to-human PK factor shown here is the RGDR for the rat nose, which in EPA methodology is applied to the BMC/BMCL as a multiplicative factor. This is unlike the PBPK-derived PK factors above, which are applied as divisors for the BMC/BMCL values.

6.3.2 2,3-Pentanedione

The ventilation coefficient[****] of 2,3-pentanedione among male mice was −0.312 ± 0.0139 and among females it was −0.182 ± 0.0530. The relative potency estimates (diacetyl/2,3-pentanedione) are shown in Table 6-10, below, and range from 0.81–7.3, depending on sex and the specific endpoint evaluated. A relative potency of 1.00 indicates that the two compounds have equal toxic potency for the endpoints examined; a relative potency less than 1.00 indicates that 2,3-pentanedione is more toxic than diacetyl, while a relative potency greater than 1.00 indicates that 2,3-pentanedione is less toxic than diacetyl. Model-based 95% confidence limits range from 0.55–14, and the overdispersion-adjusted confidence limits range from 0.44–21. These estimates suggest that the potency of diacetyl was significantly greater than that of 2,3-pentanedione among female mice for these responses. However, one source of contribution to these estimates among females is that their fitted ventilation coefficient of diacetyl exposure was 2.9-fold of the coefficient fitted for 2,3-pentanedione exposure; thus, the observed responses were associated with substantially less diacetyl having been inhaled thereby increasing its fitted potency relative to 2,3-pentanedione. In contrast the corresponding value among males was 1.2. Furthermore, all seven estimates among females depended on the modeling assumption that the exposure duration parameter was identical to that of males and results of profiling the likelihood (not shown) illustrated that this dependence was unidimensional, i.e., the seven relative potency estimates for the females varied in unison with the duration parameter, whereas this was not the case for the seven parameter estimates of the males. Hence, the interpretation of the relative potency estimates among females warrants a substantially larger degree of caution. Although the majority of the relative potency estimates among male mice are greater than 1.0, suggesting that 2,3-pentanedione may be somewhat less toxic than diacetyl, two of the seven relative potency estimates (for olfactory epithelial atrophy and respiratory epithelial degeneration in the nasal tissues of male mice) are less than 1.0. In addition to these endpoints, the overdispersion-adjusted lower confidence limit estimates of relative potency for necrosis of the nasal respiratory epithelium, chronic bronchial inflammation and bronchial epithelial regeneration are also less than 1.0. Hence, these results suggest that equal or greater toxic potency for 2,3-pentanedione relative to diacetyl cannot be ruled out on the basis of currently available data.

[****]Estimate of $\varphi_{s,PD}$ ± Model-based standard error per 100 ppm

6.4 Discussion

6.4.1 Diacetyl

6.4.1.1 Modeling issues in BMC estimation for diacetyl

Categorical regression modeling for diacetyl BMC estimation was initially conducted as described in section 6.2.2.1 for rat and mouse data. However, it was noted that the mouse models showed systematic overprediction of the observed response at the highest exposure concentrations. Mice are well known to exhibit reduced respiration when exposed to respiratory irritants [Alarie and Stokinger 1973], including diacetyl [Larsen et al. 2009]. Reduced respiratory rate and reduced minute volume have been observed in male mice exposed to diacetyl [Morgan et al. 2008]. Speculatively, reduced respiration at high exposure concentrations may contribute to the attenuation of response noted in the high exposure groups, relative to a model where the effects of exposure are proportional to concentration. A strategy was therefore employed of modifying the model structure by including a quadratic dose term parameterized

Table 6-10. Relative potency estimates for diacetyl relative to 2,3-pentanedione, on the basis of data in male and female mice

Sex	Response	Relative potency (diacetyl/ 2,3-pentanedione)	Lower confidence limit*(model-based)	Upper confidence limit* (model-based)	Lower confidence limit (OD-adjusted)†	Upper confidence limit (OD-adjusted)**
F	Bronchus, inflammation, chronic	3.7	2.0	6.7	1.4	9.6
F	Bronchus, epithelium, regeneration	4.0	2.3	7.0	1.7	9.8
F	Nasal inflammation, suppurative	4.7	3.0	7.4	2.2	9.8
F	Olfactory epithelium, atrophy	2.0	1.4	2.9	1.1	3.7
F	Nasal respiratory epithelium, Metaplasia, squamous	7.3	3.8	14	2.5	21
F	Nasal respiratory epithelium, necrosis	3.5	2.2	5.3	1.7	6.9
F	Nasal respiratory epithelium, regeneration	2.9	1.6	5.3	1.1	7.7
M	Bronchus, inflammation, chronic	1.4	1.1	1.7	0.94	2.0
M	Bronchus, epithelium, regeneration	1.3	1.1	1.6	0.95	1.8
M	Nasal inflammation, suppurative	1.6	1.3	1.9	1.2	2.1
M	Olfactory epithelium, atrophy	0.89	0.70	1.1	0.60	1.3
M	Nasal respiratory epithelium, meta-plasia, squamous	1.5	1.2	1.8	1.0	2.1
M	Nasal respiratory epithelium, necrosis	1.4	1.0	1.9	0.84	2.2
M	Nasal respiratory epithelium, regeneration	0.81	0.55	1.2	0.44	1.5

*The upper and lower confidence limits form a 95% confidence limit for the relative potency estimate.

†Upper and lower confidence limits after adjusting for overdispersion, as described in section 6.2.2.7.

to represent directly proportional changes of ventilation with concentration in modeling the mouse data, which allowed sufficient model flexibility to accommodate the attenuation of response seen in the high-dose mouse data. The resulting coefficients of ventilation for nasal and lung tissues within each sex and exposure chemical were homogeneous and subsequently pooled. Furthermore, the coefficients of male mice for each chemical were similar and the diacetyl coefficient was consistent with the observations of minute volume by Morgan et al. [2008]. However, the coefficients of the two chemicals for the females were substantially dissimilar. The seven tissue responses of each mouse were jointly analyzed because they were governed by the same ventilation coefficient. To account for correlations among the responses, random effects were included in the model thereby utilizing all of the data for the estimation of parameters common to all responses. However, the increased complexity of the model in combination with the small sample sizes and discrete responses presented challenges for assessing its fit. Residuals were calculated conditional on estimates of the random effects but interpretations of these residuals based on their having an approximately normal distribution appeared to be problematic because a systematic relationship between their skewness and concentration was apparent. However, our interpretation of these residuals as providing evidence of deviations exceeding model-based predictions is prudent and motivated the increase of the widths of the confidence intervals. However, these modifications were not necessary in modeling the rat data, and were not included in the models developed for BMC estimation with the rat data.

In the current analysis, BMC estimates for diacetyl, based on categorical regression modeling, range from 16.8–68 ppm diacetyl, and the BMCL estimates range from 10–49.9 ppm diacetyl (Tables 6-6, 6-7, and 6-8). For comparison, alternative BMC estimates based on a quantal modeling range from 14.6–78 ppm, and quantal model BMCL estimates range from 2.4–57.9 ppm. Although the central BMC estimates were similar for the quantal and categorical modeling approaches, some of the quantal model BMCL estimates are several-fold lower than any obtained using categorical modeling. It is possible that this result may be due to the inclusion of additional information — response severity, as well as incidence — in the categorical regression modeling approach, leading to narrower confidence limits in comparison to the quantal modeling results. Additional sensitivity analyses explored the sensitivity of the toxicologically-based risk assessment for diacetyl to basing the mouse-to-rat extrapolation on allometrically-scaled respiration rates rather than measured values, and to basing the animal-to-human extrapolation on RfC methodology [EPA 1994] rather than the Gloede et al. [2011] PBPK model. As described in section 6.3.1.1, varying these assumptions would have relatively modest effects on the toxicologically-based REL estimate for diacetyl. As shown in Table 6-9, the lowest candidate REL values from the various sensitivity analyses are all within a factor of ±2 of the candidate REL values from the main analysis, suggesting that the value of the toxicologically-based candidate REL is not strongly dependent on these assumptions.

6.4.1.2 Comparison with other toxicologically-based risk assessments

The numerical values of BMD estimates for diacetyl are not all directly comparable, even when based on a common response rate of 10%, because of variations in the dose units used (ppm concentration versus regional penetration versus tissue concentration). The occupational exposure limits (OELs) developed by the various authors are directly comparable, but depend in part on assumptions regarding

uncertainty factors, which may vary between studies. In contrast, the HEC estimates derived in this analysis can be directly compared to the HEC estimates that have been developed in prior risk assessments.

Earlier toxicologically-based risk assessments of diacetyl [Allen 2009; Maier 2010] have been based on the 6-week and 12-week mouse study of Morgan et al. [2008], rather than the more extensive subchronic study conducted by the NTP [2011]. Because the NTP [2011] subchronic study included data from both mice and rats and included both more dose levels and more animals per dose group than the Morgan et al. [2008] study, the NTP [2011] diacetyl study was chosen as the basis for risk assessment in this document. However, comparison of the current risk assessment findings to the results of the earlier risk assessments is instructive. The HECs derived in prior diacetyl risk assessments are summarized in Table 6-11.

The BMC_{10} HEC estimates in the current study span a range of 1.8–143.7 ppm, compared to the range of 4.5–61 ppm reported in prior diacetyl risk assessments. The $BMCL_{10}$ HEC estimates in the current study span a range of 1.4–95.9 ppm, compared to the range of 1.3–10 ppm reported in prior diacetyl risk assessments. The wider range of HEC estimates in the current study, as compared to prior analyses, is partially due to the application of animal-to-human dosimetry estimates from the Gloede et al. [2011] PBPK/CFD model, which was published subsequent to the prior risk assessments and was, obviously, not available to prior risk assessors. In addition, the current study has the benefit of a more extensive toxicological data base for diacetyl because of publication of the NTP [2011] subchronic inhalation study, and therefore includes data from more pathological endpoints than the prior analyses did.

Maier et al. [2010] conducted a risk assessment for diacetyl for the purpose of deriving an OEL. This risk assessment was based on the mouse pilot study data of Morgan et al. [2008], using BMD methodology. The authors concluded that the most sensitive endpoint in the mouse

Table 6-11. HECs (ppm atmospheric concentration) corresponding to 10% BMDs and 10% BMDLs reported in prior diacetyl risk assessments

Study	Endpoint	Dose measure	BMD_{10} HEC (ppm)	$BMDL_{10}$ HEC (ppm)
Current study, categorical regression modeling	Various (Tables 6-6, 6-7, and 6-8)	Tissue concentration	1.8 – 143.7	1.4 – 95.9
Current study, quantal modeling	Various (Table 6-9)	Tissue concentration	3.1 – 95.7	0.9 – 54.3
Maier et al. [2010]	Peribronchial inflammation	Regional penetration	6.5	1.8
Allen [2009a]	Nasal inflammation	Regional penetration	61.0	10.4
Allen [2009a]	Nasal inflammation	Tissue concentration	4.5	3.0
Allen [2009a]	Peribronchial inflammation	Regional penetration	38.6	8.3
Allen [2009a]	Peribronchial inflammation	Tissue concentration	5.1	1.3
TERA [IDFA 2008]	Peribronchial inflammation	Regional penetration	9.0	2.0

was peribronchial lymphocytic inflammation. The authors estimated a $BMDL_{10}$ of 1.98 ppm diacetyl, which they converted to a HEC of 1.8 ppm, rounded to 2 ppm. The authors concluded that a total UF of 10 was appropriate, yielding in an OEL of 0.2 ppm.

A toxicologically-based quantitative risk assessment for diacetyl was conducted by Bruce C. Allen in the reports titled "A Quantitative Risk Assessment for Diacetyl Based on Respiratory Tract Lesions in Mice" [Allen 2009a] and "Report on Model Averaging Analysis and Results for Diacetyl Mouse Data Sets" [Allen 2009b] prepared under OSHA contract number DOLQ059622303 (2009) Task Order 50. These reports served as the basis for the toxicologically-based diacetyl risk assessment in the draft NIOSH criteria document for diacetyl in 2011 but have been supplanted in the current document by an analysis of more recent data. A summary of the risk assessment extracted from these reports is included here, for comparison to the current toxicologically-based quantitative risk assessment.

The [Allen 2009a] quantitative risk assessment was based on an analysis of adverse respiratory effects in mice exposed to diacetyl by inhalation for up to 12 weeks [Morgan et al. 2008]. Adverse nasal and lung effects were observed with the latter found in the peribronchial, bronchial, and peribronchiolar regions. The Morgan et al. [2008] study was used to derive BMDs, BMDLs, and corresponding HECs, as discussed below. The responses analyzed were those most relevant to longer-term exposures, i.e., those from the subchronic portion of the study that included constant exposures of 25, 50, and 100 ppm for 6 hours/day, 5 days/week, for either 6 or 12 weeks. The 6- and 12-week data were pooled for the final analysis, based on a likelihood ratio test that indicated that the 6- and 12-week results were not significantly different. A variety of dosimetric adjustments were considered in extrapolating the results from mice to humans. The most significant of these adjustments was the choice of dose metrics, either "regional penetration" (based on the percentage of diacetyl reaching a given portion of the respiratory tract), or "tissue concentration" (based on the Morris and Hubbs [2009] PBPK model). Because the choice of dose metrics has a significant impact on the HEC, and it is not clear which dose metric is preferable, HECs derived using both dose metrics are reported in Table 6-11. An assessment completed by Toxicology Excellence for Risk Assessment (TERA) [IDFA 2008] also utilized the dose-response data of Morgan et al. [2008], and estimated HECs based on BMDLs for 10% risk, comparable to those estimated in the current analysis. TERA excluded the nasal lesions from consideration prior to their analysis, stating that the evidence of upper respiratory symptoms in humans exposed to diacetyl was inconsistent and that those symptoms lacked reliable concentration-response information. In contrast, the current assessment assumes that the dose-response relationship in a test species, rather than the lesion site, is the best criterion for choosing which endpoints to model for quantitative risk estimation. Thus, the current analysis assumes that site concordance is not a requirement because once the dose has been adequately adjusted (and ideally, once toxicodynamic considerations have been carefully considered), a valid dose-response relationship at any respiratory tract site/lesion in a test species is a reasonable basis for characterizing human risk. Additionally, exact site concordance across species would not be expected after exposure to diacetyl because of the differences in deposition of the chemical within the respiratory tracts of rodents and humans, as indicated by the PBPK model of Gloede et al. [2011]. The Gloede et al. [2011] model indicates that a much higher percentage of inhaled diacetyl reaches the bronchial and bronchiolar regions in humans than in rodents which provides a basis for the findings that diacetyl toxicity is observed primarily

in the upper respiratory tract of rodents and the lower respiratory tract of humans. TERA [IDFA 2008] estimated HECs using the EPA default methods [EPA 1994] modified by the PBPK/CFD model predictions of Morris and Hubbs [2009]. However, rather than using the relationships between the default and CFD-model-predicted scrubbing factors to define a mouse-specific estimate of airway scrubbing of diacetyl, they assumed that mice were exactly like the CFD-modeled rats (i.e., used the CFD model predictions for the rats as if they were equally relevant to mice). The TERA [IDFA 2008] risk assessment did not consider light exercise conditions, as may occur in the workplace, as these were not incorporated into the PBPK/CFD modeling of Morris and Hubbs [2009]. Moreover, for the effective dose (regional penetration) measure calculated by TERA, the default mouse ventilation rates were used. As discussed above in regard to the Allen [2009a] risk assessment, the experimentally measured ventilation rates for the Morgan et al. [2008] study were substantially greater than the EPA default values (by a factor of 3 to 5), and this would have a major impact on the HEC estimates (TERA's estimates would be about 3 to 5 times greater, because the major effect of changing the ventilation rate is on the effective dose measure, V_E/SA, rather than the scrubbing).

TERA's analysis resulted in estimates of HECs that were 9 and 2 ppm, corresponding to the estimated BMD(10) and BMDL(10), respectively, from their dose-response analysis of the peribronchial inflammation endpoint from Morgan et al. [2008]. The TERA assessment suggested that a composite uncertainty factor of 10 should be used to adjust those HECs downward to an OEL. That factor of 10 was the product of a factor of 3 for interspecies differences and another factor of 3 for human variability [IDFA 2008]. These factors of 3 are well-accepted uncertainty factors commonly used by EPA and others in risk assessment. Their recommended OEL was therefore 0.2 ppm (as an 8-hour TWA).

6.4.2 2,3-Pentanedione

Toxic potency estimation for 2,3-pentanedione is constrained by both the limited numbers of animals that have been tested and the differing exposure durations used in the diacetyl and 2,3-pentanedione studies. The currently available histopathological data for repeated exposures to 2,3-pentanedione are limited to a single study involving exposures of 2 weeks + 2 days (totaling 12 exposures per animal), in both rats and mice. The rat data and female mouse data for diacetyl are limited to a single 13-week study [National Toxicology Program 2011], so that no data on the relationship of toxicity to duration of exposure are available for the rat or the female mouse. For male mice, limited data are available from the 6- and 12-week exposures reported by Morgan et al. [2008]. Although no mouse studies are available that closely approximate the 2 week + 2 day exposure protocol used in the 2,3-pentanedione study, the 6-, 12-, and 13-week diacetyl data on male mice were used to estimate an adjustment to predict what the toxicity of diacetyl would have been in a study of the same duration as the 2,3-pentanedione study. Although a small increase of toxicity with exposure duration was fitted it was retained in the model even though it was not significant in order to account for it as a source of variation in obtaining the standard errors of the seven relative potency estimates[††††] of each sex. The resulting relative potency estimates suggest that 2,3-pentanedione may have equal or greater toxic potency than diacetyl for five of the seven responses of male mice from

[††††]For those readers acquainted with the concept of Stein estimation for adjusting a set of three or more estimates an application of a criterion of Bock [1975] to the covariance matrix of each set did not support making them.

Table 6-10. Although the responses of Table 6-10 superficially suggest that 2,3-pentanedione is or seems to be less toxic than diacetyl to female mice, these estimates are sensitively dependent on the assumption that the parameter for exposure duration is identical to that of males. Furthermore, there is a complete lack of information from these studies for assessing this assumption and profiling the likelihood indicated that the relative potency estimates of the female mice were substantially sensitive to this parameter whereas this did not hold for the estimates of the males. Hence, it would be prudent to refrain from concluding that 2,3-pentanedione is less toxic than diacetyl to female mice on the basis of the estimates of Table 6-10.

Recent data support the conclusion that 2,3-pentanedione should be used cautiously in the workplace and exposures to 2,3-pentanedione should be minimized. Rats (but not mice) develop intramural and intraluminal airway fibrosis following exposure to either diacetyl or 2,3-pentanedione [Morgan et al. 2016]. This lesion shares many features with obliterative bronchiolitis of humans, the condition that originally brought medical attention to employees exposed to diacetyl. A 2-week inhalation exposure of 150 or 200 ppm to either diacetyl or 2,3-pentanedione could produce bronchial fibrosis in rats [Morgan et al. 2016]. This finding suggests that 2,3-pentanedione causes airway fibrosis comparable to diacetyl at equal exposure concentrations. Because no chronic or subchronic studies of 2,3-pentanedione are currently available and the number of rats in the 2-week exposure is low, it is not possible to quantitatively assess the toxicity of 2,3-pentanedione relative to diacetyl for producing airway fibrosis. However, these data do suggest that it would be prudent to treat 2,3-pentanedione as at least equally toxic as diacetyl until additional toxicological data become available on the toxic potency of 2,3-pentanedione.

6.5 Conclusions

Pathological lesions produced by inhalation exposure to diacetyl and 2,3-pentanedione have been assessed using categorical regression techniques and benchmark dose estimation. For diacetyl a CFD/PBPK model is available for both rats and humans that allows rodent BMC and BMCL estimates to be extrapolated directly to human exposures. The results of this exercise indicate that the most sensitive endpoint in terms of estimated human toxicity is that associated with eosinophilic inflammation in the male rat lung. The HEC associated with this endpoint is 1.8 ppm, with a 95% lower-bound estimate of 1.4 ppm (Table 6-6). Application of a 24-fold uncertainty factor to the lower-bound HEC leads to a candidate REL of 0.06 ppm, or 60 ppb diacetyl. The estimated human toxicity based on chronic bronchial inflammation in the female mouse lung is very similar to the rat-based estimate (Table 6-8), and also leads to a candidate REL of 0.06 ppm or 60 ppb. If human data on the toxicity of diacetyl were not available, these estimates could serve as the bases for REL development for diacetyl. Because human data do exist and are sufficient for derivation of an REL, the toxicologically-based candidate RELs should be viewed as complementary to the epidemiologically-based REL. Because the toxicologically-based REL is within an order of magnitude of the epidemiologically-based REL it supports the epidemiologically-based REL.

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