Chapter 6: Quantitative Risk Assessment Based on Animal Data

6.1 Introduction

6.1.1 Diacetyl

Dose-response data for diacetyl toxicity in experimental animals are available, and there are limited but useful animal data on the toxicity of 2,3-pentanedione. For diacetyl, NIOSH has assessed these data to determine whether they support the estimate of human risk described in Chapter 5. 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: Basis of Recommended Standards for Diacetyl and 2,3-Pentanedione.

Experimental 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.

More recently the NTP has issued preliminary 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 derive benchmark doses (BMDs), the lower bound on the BMDs (BMDLs), and corresponding human equivalent concentrations (HECs), as discussed below.

6.1.2 2,3-Pentanedione

Toxicological data for 2,3-pentanedione are limited to a single 2-week pilot study using small numbers of animals [Morgan et al. 2010]. 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.

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.

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

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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. [2012b]. Individual animal data from this study were graciously provided for this analysis by Dr. Daniel Morgan, 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 below 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 animal's responses were provided by Dr. Daniel Morgan, NIEHS (personal communication to Dr. Christine Sofge, NIOSH, November 18, 2008, and November 20, 2008).

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

6.2.2 Analytical approach

6.2.2.1 Benchmark concentration analysis for rats exposed to diacetyl

Benchmark concentration 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 data, a categorical regression procedure was used to estimate benchmark concentrations. The severity scores[1] for each tissue and type of lesion were assumed to be samples from a multinomial distribution following a complementary[2] cumulative logistic model fitted separately for each species and sex as follows:

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

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

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

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.

The method of maximum likelihood was applied in order to fit[3] the model, and a likelihood ratio 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 likelihood ratio 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

[1] When no evidence of the lesion being modeled was detected a severity score of zero (0) was assigned.
[2] The term complementary discerns this model from an equivalent cumulative logistic model of $Pr(Y_{ci} < j)$.
[3] The Logistic procedure of SAS™ 9.3 was used.

by 0.10 over controls[4] 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[5]; both a two-sided 95% confidence interval and a lower one-sided 95% confidence limit (BMCL) were estimated.

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 in the fitted models indicated systematic over-prediction of the response in the high-dose groups (data not shown). Therefore a more complex modeling procedure was adopted for estimating mouse BMCs, in which a quadratic dose term was added to the model to allow the modeled response to more closely fit the data in the high-dose region of the dose-response relationship. 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 for the different durations of exposure in the various studies, were added to the model. This model was further extended to incorporate the comparative potency analysis of 2,3-pentanedione relative to diacetyl; 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

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

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

International Commission on Radiological Protection 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. The rat:human extrapolation factors used are shown in Table 6.4.

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	nose	mouth	mouth	nose + mouth	nose + mouth
Rest/exercise	rest	rest	exercise	rest + exercise*	rest + exercise*
	Human-to-rat ratio†				Human-to-mouse ratio‡
Proximal nose	1.59			0.66	0.28
Proximal trachea	4.24	6.06	7.58	8.7	2.7
Mainstem bronchi	10.00	14.00	21.00	23	7.3
Small bronchi	7.22	10.00	32.22	32	10
Average bronchi§	8.61	12.00	26.61	28	8.7
Bronchioles	5.00	7.27	40.91	40	12
Rat small bronchi to human bronchiole	0.61	0.89	5.00	4.8	3.2

*"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

‡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

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

Because a PBPK model for diacetyl exposures in the mouse is not currently available, the rat PBPK model [Gloede et al. 2011] was extended to the mouse using the USEPA 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 RGDR. The RGDR estimates the ratio of gas deposition with a given respiratory tract region in the two species being compared.

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, below. 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].

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	15	22.5	—	—
Mouse	128.5	3	3.5	2.4	3.6

*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

The RGDR is used to adjust a POD, i.e., a BMC or BMCL in the experimental 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. 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 UF – discussed below – rather than a dosimetric adjustment.

6.2.2.6 Application of uncertainty factors

The human-equivalent BMCs and BMCLs (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 √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 conditional on unobserved random effects associated with each mouse were assumed to be samples from multinomial distributions described by the following family of complementary cumulative logistic models:

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

$$= \alpha_{sjr(t)} + u_{skci} + \omega_s \cdot \tau_{skci}$$

$$+ f_{skcti}\beta_{sjr(t)}\left\{ m\left(s, k, conc_{kci}, t, \tau_{skci};\ \theta_{sr(t)}, \varphi_{skt}, \gamma_s\right)\right\} \cdot conc_{skci},$$

where s indexes sex,

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

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

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

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

$Y_{skcr(t)i}$ 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[6] except necrosis of the respiratory epithelium of the nose where the range was $\{0, 1, 2\}$,

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

$\alpha_{sjt(r)}$: $j \in \left\{1, \ldots, \max\left(Y_{kcit(r)}\right)\right\}$ denotes the intercept parameters for lesion $r(t)$ which are subject to constraints[7] $\alpha_{s3t(r)} < \alpha_{s2t(r)} < \alpha_{s1t(r)}$,

$u_{skci} \sim N(0, \sigma_{su}^2)$ is a normally distributed random effect associated with the i^{th} mouse of *skc*; likelihood ratio tests of null values of the variance parameters, σ_{us}^2, were performed and subject to being incorporated into the model.

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

$\beta_{sjr(t)}$: $j \in \left\{1, \ldots, \max\left(Y_{skcir(t)i}\right)\right\}$ are slope parameters for the effect exposure to 2,3-pentanedione, which are subject to constraints[8] $\beta_{s3r(t)} \leq \beta_{s2r(t)} \leq \beta_{s1r(t)}$ and modification by the multiplicative function,

$m\left(s, k, conc_{kci}, t, \tau_{skci};\ \theta_{sr(t)}, \varphi_{skt}, \gamma_s\right) = [1 + \gamma_s \cdot \tau_{skci}]\left[1 + I(k = 1) \cdot \left(\theta_{sr(t)} - 1\right) + \varphi_{skt} \cdot conc_{kci}\right]$ where the factor,

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

[7] These constraints derive from the requirement that $Pr\left(Y_{kcit(r)} \geq 3\right) < Pr\left(Y_{kcit(r)} \geq 2\right) < Pr\left(Y_{kcit(r)} \geq 1\right)$.

[8] These constraints derive from the requirement that $Pr\left(Y_{kcit(r)} \geq 3\right) < Pr\left(Y_{kcit(r)} \geq 2\right) < Pr\left(Y_{kcit(r)} \geq 1\right)$; furthermore, hypotheses, $\beta_{sjr(t)} = \beta_{sr(t)}\ \forall\ j \in \left\{1, \ldots, \max\left(Y_{kcit(r)}\right)\right\}$, were tested and subject to being incorporated into the model.

$[1 + \gamma_s \cdot \tau_{skci}]$, describes an adjustment for the longer durations of the diacetyl study parameterized by $\gamma_s > -1/max(\tau_{skci})$; however, the assumption, $\gamma_s = \gamma$, was imposed because data on this parameter was unavailable from female mice, the diacetyl indicator, $I(k = 1) = 1$, when $k = 1$ and $I(k = 1) = 0$ when $k = 0$, $\theta_{sr(t)}$ are parameters describing the potency of diacetyl relative to 2,3-pentanedione at low doses for $\{r(t)\}$; the hypothesis, $\theta_{sr(t)} = \theta_s$, 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 to concentration where φ_{skt} is the constant of proportionality; the hypothesis, $\varphi_{sk,lung} = \varphi_{sk,nose} = \varphi_{sk}$, was tested and subject to being incorporated into the model.

f_{skcti} 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 skc acting multiplicatively on the effect of dose. Each f_{skcti} was modeled as having unit expectation and the variance of $\log(f_{skcti}) = \sigma_{st}^2, t = 1, 2$ 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 was necessary, i.e., $f_{skc1i} \equiv f_{skc2i}$ 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 as well as facilitating residual analysis to suggest additional models for consideration. For example, evidence of a negative quadratic effect was first detected by examination of plots of residuals vs. concentration of mice. Models were fitted by the method of maximum likelihood; for models that included (unobserved) random effects the likelihood was obtained by integrating

out these effects using adaptive Gaussian quadrature[9] 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 $-2\log(Likelihood\ ratio)$. 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:

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

$$= \alpha_{sjr(t)} + \omega_s \cdot \tau_{skci} + f_{skcti}\beta_{sr(t)}\left\{ m\left(s,k,conc_{kci},t,\tau_{skci};\ \theta_{sr(t)},\varphi_{sk},\gamma\right)\right\} \cdot conc_{skci}$$

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

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

- Null values of the variance parameters, σ_{us}^2[intercept random effects omitted],
- $\beta_{s3r(t)} = \beta_{s2r(t)} = \beta_{s1r(t)} = \beta_{sr(t)}$ [single 2,3-pentanedione slope parameter for each $sr(t)$],
- Separate relative potency parameters, $\theta_{sr(t)}$ were retained since the hypothesis, $\theta_{sr(t)} = \theta_s$, was rejected; hence, $\theta_{sr(t)}\beta_{sr(t)}$ 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.

[9] The method was implemented using the NLMixed procedure of SAS® version 9.3.

Two-sided 95% confidence limits were based on application of a normal approximation to the natural logarithms of the BMCs and relative potencies where the former were associated with a 10% benchmark response for additional risk.[10] Each of the two sets of estimates was evaluated for minimax adjustment based on an extension of Stein estimation as described by Bock [1975]. Furthermore, a saturated fixed-effects model with random effects omitted[11] was fitted in order to assess the fit of 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 was used to estimate a factor for adjusting the width of the confidence intervals analogous to an adjustment for over-dispersion because the model-based confidence intervals may be too narrow if the model is incorrect.

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 Tale 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.

[10] i.e., $Pr\left(Y_{skcr(t)} \geq j | \; conc = BMC_{jskr(t)}, f_{skcti} = 1\right) - \; Pr\left(Y_{skcr(t)} \geq j | \; conc = 0, f_{skcti} = 1\right) = \; 0.10$.

[11] An attempt to include random effects in the saturated model was unsuccessful having failed to complete a single iteration after 100 hours of CPU time on a dedicated workstation.

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 which 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 which could be modeled adequately by the criteria described in section 6.2.2.7 are shown in Tables 6.7 and 6.8.

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. The criterion given by Bock [1975] supported making no minimax adjustments of these estimates.

Overall, the BMCs range from 17–68 ppm diacetyl, and the BMCLs range from 10–50 ppm diacetyl. After interspecies pharmacokinetic adjustments based on the Gloede et al. [2011] model, the human-equivalent BMCL values (BMCL_HECs) range from 1.4–96 ppm diacetyl, and the BMCL candidate REL values (after the application of uncertainty factors) range from 0.06–4.0 ppm diacetyl.

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 9.7-78 ppm, with BMCLs of 1.6-58 ppm. The $BMCL_{HEC}$ values ranged from 0.89-54 ppm, and the $BMCL_{REL}$ values ranged from 0.04-2.26 ppm, as shown in Table 6.9.

Table 6.6. Benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits (RELs) 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	0.4917	0.4543	43	30	15.7	2.7	1.9	24	0.11	0.08
M	Lung	Inflammation, eosinophil or acute	0.0549	0.3495	29	22	15.7	1.8	1.4	24	0.08	0.06
M	Nose	Olfactory epithelium, degeneration	0.5879	0.9481	20	13	0.66	30.3	19.7	24	1.26	0.82
M	Nose	Olfactory Epithelium, metaplasia, respiratory	0.6687	0.7812	41	27	0.66	62.1	40.9	24	2.59	1.70
M	Nose	Olfactory epithelium, necrosis	0.2279	0.6170	27	19	0.66	40.9	28.8	24	1.70	1.20
M	Trachea	Epithelium, hyperplasia	0.2812	0.8055	68	47	8.7	7.8	5.4	24	0.33	0.23

Table 6.6. Benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits (RELs) based on toxicity in rats exposed to diacetyl (continued)

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)
F	Nose	Inflammation, suppurative	0.1245	0.5854	22	15	0.66	33.3	22.7	24	1.39	0.95
F	Nose	Lymphoid tissue, hyperplasia	0.8265	0.1970	23	18	0.66	34.8	27.3	24	1.45	1.14
F	Nose	Turbinate, atrophy	0.4238	0.9995	36	24	0.66	54.5	36.4	24	2.27	1.52

*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.

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Table 6.7. Benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits (RELs) 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

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Table 6.8. Benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits (RELs) 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

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Table 6.9. Alternate benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits (RELs) based on dichotomizing the data, fitting quantal models, and model averaging

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	78	57.7	15.7	4.97	3.68	24	0.21	0.15
Rat	Male	Lung	Histiocytic infiltration	72.5	57.9	15.7	4.62	3.69	24	0.19	0.15
Rat	Male	Nose	Olfactory epithelium, metaplasia, respiratory	19.1	10.4	0.66	28.94	15.76	24	1.2	0.66
Rat	Male	Trachea	Epithelium, hyperplasia	52.1	21.6	8.7	5.99	2.48	24	0.25	0.10
Rat	Female	Nose	Lymphoid tissue, hyperplasia	9.7	1.6	0.66	14.70	2.42	24	0.61	0.10
Mouse	Male	Lung	Bronchus, epithelium, hyperplasia, atypical	42.4	29.4	8.7	4.87	3.38	24	0.20	0.14
Mouse	Male	Lung	Bronchus, epithelium, regeneration	49.9	41.3	8.7	5.74	4.75	24	0.24	0.20
Mouse	Male	Larynx	Chronic inflammation	16	4.7	2.7	5.93	1.74	24	0.25	0.07
Mouse	Male	Larynx	Epithelium, necrosis	11.1	4.2	2.7	4.11	1.56	24	0.17	0.07
Mouse	Male	Larynx	Squamous epithelium, hyperplasia	17.5	4.2	2.7	6.48	1.56	24	0.27	0.07
Mouse	Male	Nose	Olfactory epithelium, metaplasia	26.8	15.2	0.28	95.71	54.29	24	3.99	2.26
Mouse	Male	Trachea	Epithelium, degeneration	28.1	12.9	2.7	10.41	4.78	24	0.43	0.20
Mouse	Male	Trachea	Epithelium, hyperplasia	42.4	29.4	2.7	15.70	10.89	24	0.65	0.45
Mouse	Female	Lung	Bronchus, epithelium, regeneration	26.8	15.3	8.7	3.08	1.76	24	0.13	0.07
Mouse	Female	Larynx	Chronic inflammation	16	2.4	2.7	5.93	0.89	24	0.25	0.04
Mouse	Female	Larynx	Epithelium, necrosis	24.3	12.6	2.7	9.00	4.67	24	0.38	0.19
Mouse	Female	Larynx	Respiratory epithelium, necrosis	14.6	7.3	2.7	5.41	2.70	24	0.23	0.11
Mouse	Female	Larynx	Squamous epithelium, hyperplasia, atypical	23.8	10.5	2.7	8.81	3.89	24	0.37	0.16

6.3.2 2,3-Pentanedione

The relative potency estimates (diacetyl/PD) are shown in Table 6.10, below, and range from 0.81–7.32, depending on sex and the specific endpoint evaluated. Model-based 95% confidence limits range from 0.55–14.22, and the overdispersion-adjusted confidence limits range from 0.44–21.29. The criterion given by Bock [1975] supported making no minimax adjustments of these estimates. The potency of diacetyl was significantly greater than that of PD among female mice for these responses. However, although the majority of the relative potency estimates among male mice are greater than 1.0, suggesting that PD 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. These results suggest that equal or greater toxic potency for PD relative to diacetyl cannot be ruled out on the basis of currently available data.

Table 6.10. Relative potency estimates for diacetyl relative to PD, on the basis of data in male and female mice.

Sex	Response	Relative Potency (diacetyl/PD)	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, metaplasia, 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.

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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 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 the modeled response. A strategy was therefore employed of modifying the model structure by including a quadratic dose term in modeling the mouse data, which allowed sufficient model flexibility to accommodate the attenuation of response seen in the high-dose mouse data. This modification was not necessary in modeling the rat data, and was 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 17–68 ppm diacetyl, and the BMCL estimates range from 10-50 ppm diacetyl (Tables 6.6, 6.7, and 6.8). For comparison, alternative BMC estimates based on a quantal modeling range from 9.7–78 ppm, and quantal model BMCL estimates range from 1.6–57.9 ppm. Although the central BMC estimates were similar for the quantal and categorical modeling approaches, some of the quantal model BMCL estimates are substantially 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.

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 have been based on the 6- 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, below.

The BMC_{10} HEC estimates in the current study span a range of 1.8–144 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 1.4–96 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 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 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 below in Table 6-11.

An assessment completed by 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; therefore, it is not surprising 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, VE/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).

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 – 144	1.4 – 96
Current study, quantal modeling	Various (Table 6.9)	Tissue concentration	3.1 – 95.7	0.89 – 54
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

6.4.2 2,3-Pentanedione

Toxic potency estimation for PD is constrained by both the limited numbers of animals that have been tested and the differing exposure durations used in the diacetyl and PD studies. The currently available data for PD 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 male mouse studies are available that closely

approximate the 2 week + 2 day exposure protocol used in the PD study, it is possible to use the 6-, 12-, and 13-week diacetyl data to estimate what the toxicity of diacetyl would have been in a study of the same duration as the PD study. The resulting relative potency estimates suggest that PD may have equal or greater toxic potency than diacetyl for five of the seven responses of Table 6.10.

The additional data, though preliminary in nature, suggest that PD should be used cautiously in the workplace and exposures to PD should be limited. Rats (but not mice) develop intramural and intraluminal airway fibrosis following exposure to PD [Morgan et al. 2012b]. This lesion shares many features with bronchiolitis obliterans of humans, the condition that originally brought medical attention to workers exposed to diacetyl. In a follow-up study, currently published only in abstract form, a 2-week inhalation exposure to either diacetyl or PD could produce intramural or intraluminal fibrosis in rats [Morgan et al. 2012a]. In that study, the percentage of rats with airway fibrosis was higher in the PD exposed rats than in the diacetyl exposed rats. This finding, though based on very limited data, may suggest that PD is more toxic to the lung than diacetyl at equal exposure concentrations. Because no chronic or subchronic studies of PD 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 PD relative to diacetyl for producing airway fibrosis. However, these data do suggest that it would be prudent to treat PD as at least equally toxic as diacetyl until additional toxicological data become available on the toxic potency of PD.

6.5 Conclusions

Pathological lesions produced by inhalation exposure to diacetyl and PD have been assessed using categorical regression techniques and benchmark dose estimation. For diacetyl a CFD/PBPK model is available for both rats and humans which 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 a 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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