Gas exposures are characterized by concentration ($mg/m^3$), temperature, and pressure. If the concentration is expressed in ppm, the actual temperature and pressure should be used to convert the units to $mg/m^3$. When the actual temperature and pressure values are not provided in a study, it should be suspect for deficient reporting of important experimental detail. Some studies, however, express values already corrected for these parameters, usually corrected to 25 °C and 760 mm Hg. These values are the recommended default values for temperature and pressure, respectively.

Other assumptions and default values for gas and vapor extrapolations are provided in Appendix J.

### 4.3.7 Derivation and Dosimetric Adjustment Using Human Studies

Whenever possible, a human study is preferred as the critical study for derivation of an RfC. This avoids the problems of extrapolating from laboratory animals to humans, but has its own limitations. When using epidemiologic data to assess risk in the context of a method designed for data on experimental animals, the dependence of epidemiologic studies on existing exposure conditions and the necessity of using noninvasive diagnostic methods present two complicating factors. One is that existing exposure levels may not include a NOAEL. Toxicologic studies are generally designed to identify the NOAEL. For ethical reasons, many clinical studies in humans often focus on exposure scenarios that are associated with minimal effects and short exposure durations, although they also may identify a NOEL. In contrast, epidemiologic studies cannot be designed as rigorously because exposure levels are dependent on existing exposures. Furthermore, often exposures in epidemiological studies are poorly characterized. In both controlled human and animal studies, the effect level estimates are biased by the dose or exposure level selected or available for study. These effect level estimates are subject to random error, the magnitude of which depends on various design aspects, such as the size of the study population or test groups, and the underlying variability of the test animals or study subjects.

The second factor to consider for epidemiological studies is that a broad spectrum of potential adverse effects cannot be evaluated; therefore, it is difficult to determine the critical effect. Prospective epidemiologic studies that investigate an array of likely biological markers or preclinical endpoints are better sources of NOAELs/LOAELs to estimate the

threshold region. Clinical studies may be based on low exposure levels selected by the investigator and investigate sensitive endpoints, but these studies are generally of short duration and unless mechanisms of action are unequivocally established, are probably more useful for estimating short-term effects or to identify potential target tissues for consideration when evaluating chronic data. The following discussion describes approaches to address the use of human data for RfC derivation.

#### 4.3.7.1 Selecting the Threshold Estimate

In some epidemiologic studies, only severe effects such as mortality are examined so that the concept of a NOAEL in inappropriate for RfC derivation. A study in which sensitive endpoints are evaluated may identify a LOAEL but not a NOAEL. If the effect is sensitive (i.e., it occurs early in the natural history of the disease), a LOAEL may be judged suitable for use in calculating an RfC in lieu of a NOAEL, because the uncertainty of extrapolating human data for a well-defined critical effect from a LOAEL to a NOAEL is judged to be less than the uncertainty involved in extrapolating from animal data to humans. The circumstances governing this selection include deficiency in toxicologic and physiologic data bases, small sample size in the experimental studies, or physiologic or pharmacokinetic data suggesting that animal data are unlikely to be good predictors for humans.

#### 4.3.7.2 Defining the Exposure Level

Epidemiologists cannot control the exposure levels for a study in a systematic fashion, but instead attempt to estimate or measure the levels to which the study population is exposed, insofar as is possible for that study. In actual exposure situations, the levels vary in time and location. Epidemiologic studies can utilize a variety of parameters to characterize exposure, although in retrospective studies the available data are usually quite limited.

The ideal exposure measure for humans who move about in their environment is individual data, such as might be obtained with the use of a personal monitor. However, in addition to the expense and practical difficulties, this technology is available for measuring only a few chemicals. Individual exposure can be constructed by mapping the individual's time in various exposure zones, rooms, or areas. If information on levels in the environment

is not available, duration of employment in a particular job category often is used as a surrogate for exposure.

Parameters commonly used to measure environmental levels are cumulative exposure, peak exposure level, time-weighted average, and ratio of average to peak exposure. Currently, it is unclear which of these is best related to disease. For example, cumulative exposure is more appropriate as the half-life of a substance is increased. Therefore, to derive RfCs that identify levels of environmental exposures free of adverse effects, cumulative exposure or time-weighted averages are appropriate for substances with long half-lives. The circumstances must be evaluated on a case-by-case basis and different exposure parameters may be used if the rationale is presented. For conversion of units, the approach is the same as that for laboratory animal data (Equations 4-1a and 4-1b). Considerations for route-to-route extrapolation would be the same as for laboratory animal data; however, it is highly unlikely that human ingestion data would be available in a form useful for quantitative derivation of an RfC.

#### 4.3.7.3 Dosimetric Adjustment for Human Data

When human data are available and adequate to derive an RfC, adjustments are usually required to account for differences in exposure scenarios (e.g., extrapolation from an 8 h/day occupational exposure to a continuous chronic exposure). The optimal approach is again to use a biologically motivated mathematical or PBPK model. An occupational exposure can be extrapolated in the same fashion as described in Section 4.3.3 to extrapolate intermittent exposure regimens from experimental laboratory animals, using particle deposition or PBPK models with human exertion (work) ventilation rates and exposure durations appropriate to the occupational setting.

In the event that a PBPK model or required physicochemical and physiological parameters are not available, the default approach for human exposure scenarios is to adjust by the default occupational ventilation rate and for the intermittent work week schedule:

$$NOAEL^{*}_{[HEC]} = NOAEL\ (mg/m^3) \times (VEho/VEh) \times 5\ days\ /\ 7\ days \qquad (4\text{-}49)$$

where:

$NOAEL^{*}_{[HEC]}$ = the NOAEL or analogous effect level obtained with an alternative approach as described in Appendix A, dosimetrically adjusted to an ambient human equivalent concentration;

NOAEL = occupational exposure level (time-weighted average);

VEho = human occupational default minute volume (10 $m^3$/8 h); and

VEh = human ambient default minute volume (20 $m^3$/24 h).

#### 4.3.7.4 Uncertainty Factors for Human Data

Areas of extrapolation and the UFs applied to account for them are essentially the same as those for extrapolating laboratory animal data described in Section 4.3.8. The use of human data, in most cases, will obviate only the use of the UF for interspecies extrapolation. The best data to use for calculating an RfC would be a population study of humans that includes sensitive individuals exposed for lifetime or chronic duration, and that evaluates the critical endpoint or an appropriate early marker for the disease. A NOAEL derived from a well done epidemiologic study of this description may require no UF. A similar study in humans that contains only a LOAEL would require the use of a factor of up to 10-fold to reduce the exposure to the range of a NOAEL. Chronic studies on populations that do not include sensitive individuals may require a 10-fold UF. For example, studies of workers are considered to contain only relatively healthy adults. A NOAEL from a study that entails subchronic exposure would require a reduction by a 10-fold UF. However, the amount of exposure in a human study that constitutes subchronic is not defined, and could depend on the nature of the effect and the likelihood of increased severity or greater percent response with duration. In the absence of data on the relationship of animal to human lifespan for predicting health effects, a linear correlation of percent lifespan is sometimes assumed. For example, because a study in animals that is 10% of lifespan is considered subchronic, then 7 years or one-tenth of the assumed human lifetime (70 years) is used as interim guidance for the superfund program to determine the working cut-off for deriving a subchronic human study (Means, 1989). Information on the natural history and progression for the disease should be considered and explained; information on follow-up after exposure, often available in epidemiologic studies, is important.

In some cases, short-term studies of effects in humans can give important information on irritation, sensory effects, or sensitivity and reversibility, yet give no information on the effect of chronic exposure.

### 4.3.8 Data Array Evaluation and Choice of Principal Study/Studies

Inhalation reference concentrations are typically calculated using a single exposure level and UFs that account for specific deficiencies in the toxicity data base. Both the exposure level and the UFs are selected and evaluated in the context of all available chemical-specific literature. After all toxicological, epidemiologic, and supporting data have been reviewed and evaluated, a principal study (or studies) is selected that reflects optimal data on the critical effect. Dose-response data points for all reported effects are examined as a component of this review. Issues of particular significance in this endeavor include

- A delineation of all toxic effects and associated exposure levels (see Section 4.2).
- Dosimetric adjustment to HEC (see Section 4.3).
- Determination, to the extent possible, of effect-specific experimental threshold regions (i.e., the $NOAEL_{[HEC]}$-$LOAEL_{[HEC]}$ interface or bracket).
- Determination of the critical effect. Of the multiple toxic endpoints potentially observed, the critical effect selected is defined as the one associated with the lowest $NOAEL_{[HEC]}$-$LOAEL_{[HEC]}$ interface or bracket.
- Special consideration of species, portal-of-entry effects, and/or route-specific differences in pharmacokinetic parameters and the slope of the dose-response curve.

If multiple $NOAEL_{[HEC]}$s for the same critical effect are available in one animal species, the highest $NOAEL_{[HEC]}$ for that individual species is compared to $NOAEL_{[HEC]}$s for that effect from other species. If multiple $NOAEL_{[HEC]}$s for the critical effect are available in different species, the lowest of these $NOAEL_{[HEC]}$s, or the $NOAEL_{[HEC]}$ for the most sensitive species, generally is selected as the exposure level that most closely defines the threshold for adverse effects of the dose-response curve. When disparity in dose-response patterns is apparent between species, studies need to be evaluated to ascertain, if possible,

whether the differences are due to (1) differences in the monitored endpoints or procedures across studies, (2) species differences in dose-respone curves, or (3) choice of dose-spacing (if alternative approaches such as the benchmark or Bayesian approaches described in Appendix A are not used). If species differences are apparent, the question arises as to which species is the most appropriate model for humans. Differences in dose-effect curves could be due to inherent differences in target receptor sensitivity (pharmacodynamics) or to differences in concentration of the compound or metabolite reaching the receptor (pharmacokinetics). This distinction is important when trying to identify the most appropriate species for modeling the human response. Current controversy with respect to the URT in the area of data array analysis involves the relevance of nasal lesions in laboratory rodents versus humans or other primates (DeSesso, 1993) and whether nasal lesions in rodents are somehow sentinel for effects in the lower respiratory tract of primates (Jarabek, 1994). It is consistent with EPA policy to use data on the most sensitive animal species as a surrogate to humans unless data exist to the contrary. In the RfC methodology, this evaluation is based on $NOAEL_{[HEC]}$s.

Often an appropriate $NOAEL_{[HEC]}$ will not be available. In that event, other estimates of effect-specific thresholds may be used. Based on the dose-effect classification system presented in Tables 4-2 and 4-3, the following guidelines may be employed (adapted from Federal Register, 1980):

- An $FEL_{[HEC]}$ from a study with no other dose-response levels (a free-standing $FEL_{[HEC]}$) is unsuitable for the derivation of an RfC.
- A $NOEL_{[HEC]}$ from a study with no other dose-response levels is unsuitable for the derivation of an RfC. If multiple $NOEL_{[HEC]}$s are available without additional data, $NOAEL_{[HEC]}$s, or $LOAEL_{[HEC]}$s, the highest $NOEL_{[HEC]}$ should be used.
- A $LOAEL_{[HEC]}$ from a study with no other dose-response levels (a free-standing $LOAEL_{[HEC]}$) is unsuitable for the derivation of an RfC.
- A $NOAEL_{[HEC]}$ or $LOAEL_{[HEC]}$ supported by other data may be suitable for RfC derivation. In the case of a $LOAEL_{[HEC]}$, an additional UF is applied for extrapolation to NOAEL.

*Note: Caution must be exercised not to substitute a $FEL_{[HEC]}$ for a $LOAEL_{[HEC]}$.*

- If, for reasonably closely spaced doses, only a $NOEL_{[HEC]}$ and a $LOAEL_{[HEC]}$ of equal quality are available, then the appropriate uncertainty factor is applied to the $NOEL_{[HEC]}$.

In the course of many risk assessment discussions during the last several years, the EPA has decided on the following conditions when choosing the appropriate animal effect or no-effect level as a basis of an RfC. If an appropriate human study with a well-defined $NOAEL_{[HEC]}$ is available as to a chemical's critical effect, it is used in preference to laboratory animal toxicity data in estimating RfCs. When such human data are not available, the following sequence is used to choose the appropriate study, species and $NOAEL_{[HEC]}$ as a basis of RfC estimation.

- The EPA chooses the most appropriate $NOAEL_{[HEC]}$ of the critical effect from a well-conducted study on a species that is known to resemble the human in response to this particular chemical (e.g., by comparative pharmacokinetics).

- When the above condition is not met, the EPA generally chooses the most sensitive study, species, and $NOAEL_{[HEC]}$, as judged by an interspecies comparison of the $NOAEL_{[HEC]}$ and $LOAEL_{[HEC]}$. Table 4-7 outlines examples of this condition.

### 4.3.9 Operational Derivation of the Inhalation Reference Concentration

Choice of the effect and its associated concentration that serves as the basis for derivation of the RfC requires the evaluation of the entire data array of $NOAEL_{[HEC]}$s and $LOAEL_{[HEC]}$s. An example data array is shown in Figure 4-11. The critical toxic effect to be used in the dose-response assessment is generally the one characterized by the lowest $NOAEL_{[HEC]}$ that is representative of the threshold region for the data array. For example, note in Figure 4-11 that as concentration increases above the NOAEL, the incidence or severity of the observed toxicity is also increasing. The objective when analyzing such a data array is to select a prominent toxic effect that is pertinent to the chemical's mechanism of action and which is at or just below the threshold for the relatively more serious effects. This approach is based, in part, on the assumption that if the critical toxic effect is prevented, then all toxic effects are prevented. The determination of the critical toxic effect from all effects in the data array requires toxicologic judgment because a chemical may elicit more

## TABLE 4-7. COMPARISON OF THE HIGHEST INDIVIDUAL SPECIES $NOAEL_{[HEC]}$ AND ITS $LOAEL_{[HEC]}$[a]

| Effect Level ($mg/m^3$) | Species | | | Comments (Given the Same Critical Effect) |
|---|---|---|---|---|
| | Dog | Rat | Mouse | |
| Example 1: | | | | |
| $LOAEL_{[HEC]}$ | 100 | 120 | - | The proper choice is generally the highest dog $NOAEL_{[HEC]}$ of 50 $mg/m^3$, since the potential experimental threshold in dogs (i.e., the potential $LOAEL_{[HEC]}$) may be below the highest $NOAEL_{[HEC]}$s in both rats and mice. |
| $NOAEL_{[HEC]}$ | 50 | 60 | 80 | |
| Example 2: | | | | |
| $LOAEL_{[HEC]}$ | 120 | 100 | 90 | The proper choice is generally the mouse $LOAEL_{[HEC]}$ of 90 $mg/m^3$, since the potential experimental threshold in mice may be lower than the highest $NOAEL_{[HEC]}$s for both dogs and rats. Judgment is needed in this example to ensure that the adverse effects seen in all three species are truly minimal. For example, if any of the $LOAEL_{[HEC]}$s in the species represented an increase in a severe effect, no firm basis for the development of an RfC exists. This is based on the general observation that overt toxicity data are far removed quantitatively from chronic $LOAEL_{[HEC]}$s and $NOAEL_{[HEC]}$s, and thus, the data base has failed to establish the likely experimental threshold for the most sensitive endpoint. |
| $NOAEL_{[HEC]}$ | 90 | 75 | - | |
| Example 3: | | | | |
| $LOAEL_{[HEC]}$ | 75 | 80 | 90 | The proper choice is generally the dog $LOAEL_{[HEC]}$ of 75 $mg/m^3$, since by definition this represents the most sensitive species (see, however, the caution in Example 2). |
| $NOAEL_{[HEC]}$ | - | - | - | |
| Example 4: | | | | |
| $LOAEL_{[HEC]}$ | - | - | - | The proper choice is generally the highest rat $NOAEL_{[HEC]}$ of 90 $mg/m^3$, since no assurance exists that the experimental threshold in rats is not below the highest $NOAEL_{[HEC]}$s of both dogs and mice. This situation is unusual and should be judged carefully; since a $LOAEL_{[HEC]}$ has not been determined, the RfC may be unduly conservative. Strict interpretation of this example might lead to strikingly lower RfCs if other species are tested at much lower doses. Such RfCs may not be appropriate. |
| $NOAEL_{[HEC]}$ | 100 | 90 | 120 | |

[a]$NOAEL_{[HEC]}$ or $LOAEL_{[HEC]}$ refers to NOAEL or LOAEL concentrations adjusted for dosimetric differences between laboratory animals and humans to human equivalent concentrations (HECs).

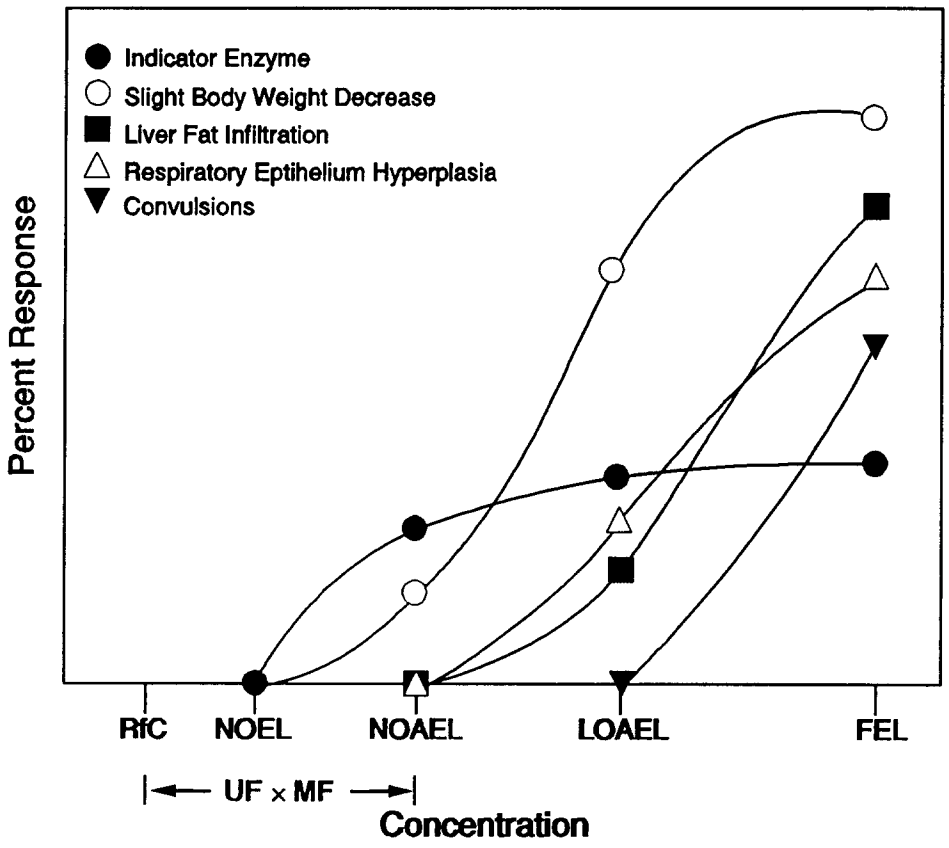


**Figure 4-11. Example data array and inhalation reference concentration (RfC) derivation.**

than one toxic effect (endpoint) in tests of the same or different exposure duration, even in one test species. Further, as discussed in Appendix A, the $NOAEL_{[HEC]}$ and $LOAEL_{[HEC]}$ obtained from studies depend on the number of laboratory animals or human subjects examined and on the spacing of the exposure levels. The $NOAEL_{[HEC]}$ (or $LOAEL_{[HEC]}$ as discussed above) from an individual study (or constellation of studies), that is also representative of the threshold region for the overall data array is the key datum synthesized from an evaluation of the data array. The study from which this $NOAEL_{[HEC]}$ (or $LOAEL_{[HEC]}$ as discussed above) is estimated is known as the principal study. Determination of the critical effect for the entire data array and identification of the principal study represents the first scientific evaluation of the dose-response analysis per se. The second is the selection of uncertainty factors and operational derivation of the estimate.

#### 4.3.9.1 Application of Uncertainty Factors[4]

The RfC is a benchmark estimate that is derived from the $NOAEL_{[HEC]}$ for the critical effect by consistent application of UFs. The UFs are applied to account for recognized uncertainties in the extrapolations from the experimental data conditions to an estimate appropriate to the assumed human scenario. Determination of which UFs to apply and the magnitude of each represents the second scientific evaluation required for an RfC dose-response assessment. The standard UFs applied are those for the following extrapolations (as required): (1) data on effects of average healthy humans to sensitive humans; (2) laboratory animal data to humans; (3) studies of subchronic to chronic duration; (4) a $LOAEL_{[HEC]}$ to a $NOAEL_{[HEC]}$; and (5) from an incomplete to complete data base. The UFs are generally an order of magnitude, although incorporation of dosimetry adjustments or other mechanistic data has routinely resulted in the use of reduced UFs for RfCs. The composite UF applied to an RfC will vary in magnitude depending on the number of extrapolations required. An RfC will not be derived when use of the data involve greater than four areas of extrapolation, however. The composite UF when four factors are used generally is reduced from 10,000 to 3,000 in recognition of the lack of independence of these factors. This coalescing of several areas of uncertainty is based on the knowledge that each individual factor is generally conservative from the standpoint of the behavior of the average chemical (Dourson and Stara, 1983), and that the multiplication of four or five values of 10 is likely to yield unrealistically conservative RfCs.

An additional modifying factor (MF) may also be applied when scientific uncertainties in the study chosen for derivation are not explicitly addressed by the standard UFs. For example, an MF might be applied to account for a statistically minimal sample size or for poor exposure characterization.

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[4]Other authors have discussed these areas of uncertainty or UFs in general. The reader is referred to Zielhuis and Van der Kreek (1979) for a discussion of these factors in setting health-based permissible levels for occupational exposure, and Dourson and Stara (1983) for a summary of these factors regarding oral exposures. Other publications include Gaylor (1983), who discusses the use of safety factors for controlling risk; Crump (1984), who discusses problems with the current methods that includes UFs; Krewski et al. (1984), who contrast safety factors and mathematical models as methods for determining "safe" levels of exposure; Calabrese (1985), who discusses UFs and interindividual variation; and Lu (1983, 1985b), who discusses safety factors from the perspective of the World Health Organization. Lewis et al. (1990) have proposed an operational alternative approach. Renwick (1991) has outlined a flexible scheme based on the nature of toxicity, knowledge of metabolism, and information of human heterogeneity.

Thus, notationally, the RfC is defined as:

$$RfC = NOAEL^{\star}_{[HEC]} / (UF \times MF), \quad (4\text{-}50)$$

where:

$NOAEL^{\star}_{[HEC]}$ = The NOAEL or analogous effect level obtained with an alternate approach as described in Appendix A, dosimetrically adjusted to an HEC;

UF = Uncertainty factor(s) applied to account for the extrapolations required from the characteristics of the experimental regimen; and

MF = Modifying factor to account for scientific uncertainties in the study chosen as the basis for the operational derivation.

It must be emphasized that the RfC as a quantitative dose-response estimate is not numeric alone. As risk assessments have become a more prevalent basis for decision-making, their scientific quality and clarity have gained unprecedented importance (American Industrial Health Council, 1989; National Research Council, 1994). Due to the complexity of many risk assessments, desirable attributes include the explicit treatment of all relevant information and the expression of uncertainty in each element (i.e., hazard identification, dose-response assessment, exposure assessment, and risk characterization). Any dose-response assessment, such as the RfC, has inherent uncertainty and imprecision because the process requires some subjective scientific judgment, use of default assumptions, and data extrapolations.

A complete dose-response evaluation should include communication of the rationale for data selection, the strengths and weaknesses of the data base, key assumptions, and resultant uncertainties (Habicht, 1992; American Conference of Governmental Industrial Hygienists, 1986). The rationale for the choice of the data from which the RfC is derived, a discussion of data gaps, and the resultant confidence in the RfC are all outlined on the summary of the RfC entered on the EPA's Integrated Risk Information System (IRIS). A discussion and rationale for the uncertainty factors used in the RfC derivation are also provided. This information is an important part of the RfC and must be considered when evaluating the RfC as a dose-response estimate, in addition to assumptions and resultant uncertainties inherent in an exposure assessment, when attempting to integrate the assessments into a risk

characterization. Additional guidance on the assignment of confidence levels is provided in Section 4.3.9.2.

Uncertainty factors are associated with various specific recognized uncertainties in extrapolating from the type of study serving as the basis for the RfC to the scenario of interest for the risk assessment as outlined in Table 4-8. The processes thought to be encompassed by each factor are provided in Table 4-9.

An additional MF may be used to account for uncertainties in the study chosen for derivation. For example, a MF may be applied to account for a study of statistically minimal sample size or with poor exposure characterization. The effect of small sample size has long been recognized in toxicology (Bliss, 1938), and recent research has focused on adjusting for this by taking the power of individual studies into account (Brown and Erdreich, 1989). Considerations of the sensitivity of the NOAEL/LOAEL approach to sample size and dose spacing has led to the development of the alternative approaches to derivation discussed in Appendix A.

In general, the choice of UFs applied reflects the uncertainty associated with estimation of an RfC from different human or laboratory animal toxicity data bases. When sufficient human data are available on a chemical's critical effect and pharmacokinetics, the UFs may be smaller than those described in Table 4-8, or unnecessary. For example, if sufficient data from chronic duration exposure studies are available on the threshold region of a chemical's critical toxic effect in a known sensitive human population, then the UF used to estimate the RfC may be 1. That is, these data are judged to be sufficiently predictive of a human population subthreshold dose, so that additional UFs are not needed. Likewise, in cases where data do not completely obviate the uncertainty for which a given UF is applied, or appears to be intermediate in fulfilling that requirement, an intermediate UF is suggested to estimate the RfC (Federal Register, 1980). Composite factors are sometimes applied to account for partial uncertainty under more than one UF. For example, a 10-fold factor may be applied to account for partial uncertainty due to both the use of less than chronic data and a LOAEL, if data supported that the effect was of minimal severity and the lesion did not progress significantly with duration. When a single subchronic study that does not define a NOAEL is the only available information, the EPA recognizes that all five areas of

## TABLE 4-8. GUIDELINES FOR THE USE OF UNCERTAINTY FACTORS IN DERIVING INHALATION REFERENCE CONCENTRATION (RfC)

| | | |
|---|---|---|
| Standard Uncertainty Factors (UFs) | | |
| H | Human to sensitive human | Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. |
| A | Animal to human | Use an additional threefold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of average healthy humans. Use of a 3 is recommended with default dosimetric adjustments. More rigorous adjustments may allow additional reduction. Conversely, judgment that the default may not be appropriate could result in an application of a 10-fold factor. |
| S | Subchronic to chronic | Use up to an additional 10-fold factor when extrapolating from less than chronic results on experimental animals or humans when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. |
| L | $LOAEL_{[HEC]}$ to $NOAEL_{[HEC]}$ | Use up to an additional 10-fold factor when deriving an RfC from a $LOAEL_{[HEC]}$, instead of a $NOAEL_{[HEC]}$. This factor is intended to account for the uncertainty in extrapolating from $LOAEL_{[HEC]}$s to $NOAEL_{[HEC]}$s. |
| D | Incomplete to complete data base | Use up to a 10-fold factor when extrapolating from valid results in experimental animals when the data are "incomplete". This factor is intended to account for the inability of any single animal study to adequately address all potential endpoints at various critical life stages. Unless a comprehensive array of endpoints is addressed by the data base, there is uncertainty as to whether the critical effect chosen for RfC derivation is the most sensitive or appropriate. |
| Modifying Factor (MF) | | |
| | | Use professional judgment to determine whether another uncertainty factor (MF) that is $\leq 10$ is needed. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above (e.g., the number of animals tested or quality of exposure characterization). The default value for the MF is 1. |

NOTE: Assuming the range of the UF is distributed lognormally, reduction of a standard 10-fold UF by half results in a UF of approximately 3 (i.e., $10^{.5}$). Composite UF for derivation involving four areas of uncertainty is 3,000 in recognition of the lack of independence of these factors. Inhalation reference concentrations are not derived if all five areas of uncertainty are invoked.

## TABLE 4-9. THE USE OF UNCERTAINTY FACTORS IN DERIVING AN INHALATION REFERENCE CONCENTRATION

| Standard Uncertainty Factors (UFs) | Processes Considered in UF Purview |
|---|---|
| H = Human to sensitive human<br>Extrapolation of valid experimental results from studies using prolonged exposure to average healthy humans.<br>Intended to account for the variation in sensitivity among the members of the human population. | Pharmacokinetics/Pharmacodynamics<br>Sensitivity<br>Differences in mass (children, obese)<br>Concomitant exposures<br>Activity pattern<br>Does not account for idiosyncracies |
| A = Animal to human<br>Extrapolation from valid results of long-term studies on laboratory animals when results of studies of human exposure are not available or are inadequate.<br>Intended to account for the uncertainty in extrapolating laboratory animal data to the case of average healthy humans. | Pharmacokinetics/Pharmacodynamics<br>Relevance of laboratory animal model<br>Species sensitivity |
| S = Subchronic to chronic<br>Extrapolation from less than chronic exposure results on laboratory animals or humans when there are no useful long-term human data.<br>Intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. | Accumulation/Cumulative damage<br>Pharmacokinetics/Pharmacodynamics<br>Severity of effect<br>Recovery<br>Duration of study<br>Consistency of effect with duration |
| L = LOAEL to NOAEL<br>Derivation from a LOAEL instead of a NOAEL.<br>Intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. | Severity<br>Pharmacokinetics/Pharmacodynamics<br>Slope of dose-response curve<br>Trend, consistency of effect<br>Relationship of endpoints<br>Functional vs. histopathological evidence<br>Exposure uncertainties |
| D = Incomplete to complete data<br>Extrapolation from valid results in laboratory animals when the data are "incomplete".<br>Intended to account for the inability of any single laboratory animal study to adequately address all possible adverse outcomes in humans. | Quality of critical study<br>Data gaps<br>Power of critical study/supporting studies<br>Exposure uncertainties |

uncertainty are present, and an RfC will not be derived. An RfC will also not be derived in the absence of data on the potential respiratory tract toxicity.

It should be noted that the basis for the UFs is empirical and has been derived from oral data (Dourson and Stara, 1983). In most cases, support of each UF was based on analysis of the ratios of effect levels. For example, analysis of the ratios of NOAELs from 90-day

studies compared to NOAELs from chronic studies was used to support a 10-fold factor to account for subchronic to chronic extrapolation. Because the different types of toxicity (portal-of-entry versus remote) may have different determinants underlying ethe exposure-dose-response continuum for which the default dosimetry adjustments only partially account, the appropriate magnitude for these UFs when using inhalation data is a topic of ongoing research at the EPA. Estimation procedures that are not sensitive to the spacing of exposure concentrations such as the benchmark and Bayesian approaches discussed in Appendix A are being explored instead of the previously used ratios approach for this research.

A UF is generally used to calculate RfCs with appropriate chronic human data, and is intended to account for intraspecies human variability to the adverse effects of a chemical (i.e., H in Tables 4-8 and 4-9). Empiric support for a 10-fold value for this UF is based on analyses of single-dose oral data (Weil, 1972; Dourson and Stara, 1983). Hattis et al. (1987) also suggest that a value of 10 is generally appropriate for this UF based on an analysis of human variability for key pharmacokinetic parameters.

For derivation of the RfC, the UF applied for interspecies extrapolation (i.e, A in Tables 4-8 and 4-9) is 3 due to the incorporation of dosimetric adjustments. If more rigorous adjustments can be made, an additional reduction of the UF would be warranted. The threefold factor represents the reduction of the usual 10-fold factor by half (i.e., $10^{.5}$) since the default dosimetry accounts for variability in disposition (pharmacokinetics). The residual uncertainty is envisioned to address species differences in pharmacodynamics. A similar scheme was proposed by Renwick (1991), although the dosimetry adjustments in the RfC methods explicitly address disposition of particles and gases via inhalation. The empiric basis of this UF was originally based on oral data (Dourson and Stara, 1983). An analysis by Jarabek and Hasselblad (1991) showed that the deviation across species and chemicals for HEC estimates was reduced approximately 2-fold versus that using previous (Federal Register, 1980) derivation methods. The dosimetric adjustments have also been shown to be consistently less than those calculated with previous methods so that a reduction in the UF was further supported (Jarabek et al, 1989; Overton and Jarabek, 1989a,b).

An RfC based on a $NOAEL_{[HEC]}$ with satisfactory subchronic laboratory animal data would require a factor to address the uncertainty in extrapolating data from subchronic to chronic exposures (i.e., S in Table 4-8). Empirical evidence supporting the proposition that

subchronic toxicity data can be used with a 10-fold UF is again based on analyses of oral toxicity data (Dourson and Stara, 1983; Weil and McCollister, 1963; Weil et al., 1969). McNamara (1976) also demonstrated that a 10-fold factor applied to a subchronic NOEL would predict a chronic NOEL for 95% of the 122 compounds for which both chronic and subchronic data for the oral route of exposure were available. To the degree that route-specific and duration-specific data are not available, increased reliance on additional extrapolation assumptions and a larger UF is necessary. The lack of data with appropriate duration becomes of greater concern when either the chemical itself or its damage has the potential to accumulate. Conversely, if the effect is more dependent on concentration than duration, and progression of the lesion (either in incidence or severity) is not evident, a reduced UF may be considered.

Generally, a UF is applied to estimate RfCs using LOAELs if NOAELs are unavailable (i.e., L in Tables 4-8 and 4-9). This UF is employed to define an exposure level below the LOAEL expected to be in the range of a NOAEL. The empiric support for this UF was based on frequency analyses of LOAEL to NOAEL ratios for oral toxicity data after either subchronic or chronic exposures (Dourson and Stara, 1983; Weil and McCollister, 1963). In practice, this UF has varied and its value is chosen based on the severity of the adverse effect of the LOAEL. For example, if the LOAEL represents liver cell necrosis, a higher value is suggested for this UF than would be suggested if the LOAEL were based on fatty infiltration because the hypothesized NOAEL should be closer to the less severe LOAEL (Dourson and Stara, 1983).

Under some circumstances, a UF is applied when the data base is deficient in comprehensiveness; for example, if it lacks a two-generation reproductive study (i.e., D in Tables 4-8 and 4-9). The rationale for the minimum data base criteria provided in Section 4-1 can provide guidance on when a UF for lack of comprehensiveness is warranted. Dourson et al. (1992) have shown this to be an appropriate factor for oral data. The requirement for data in a second species is also supported by analyses that have shown lack of concordance for target tissues across species (Appelman and Feron, 1986; Heywood, 1981, 1983). The U.S. Food and Drug Administration has addressed the data base deficiencies issue with the use of a twofold safety factor. Therefore, in situations where a subchronic animal bioassay was available, but information in a second experimental species was lacking,

a 2,000-fold safety factor (i.e., $2_D \times 10_H \times 10_A \times 10_S$) was used to estimate an acceptable daily intake (Shibko, 1981). The influence that the requirement for portal of entry data and dosimetric adjustments used in the RfC methods may have on this UF has not yet been quantified.

There are certain circumstances specific to inhalation that may require changes in UFs. For example, the UF used when extrapolating from a subchronic to a chronic study is assumed to be adequate for oral studies in the great majority of cases. A UF of extrapolation of subchronic to chronic exposures for inhalation studies also should be adequate with certain exceptions. Possible exceptions include the following:

- Exposure to chemicals that are considered likely to induce hypersensitivity (see Section 2.1.2.3),
- Exposure to chemicals that are considered likely to induce very slowly developing ("smoldering") effects (e.g., beryllium), and
- Exposure to inhaled relatively insoluble particulate matter where the clearance rate may slow or stop when a threshold for clearance is reached. Therefore, after long-term exposure, lung loads can reach much higher levels than could reasonably be expected from lower level, chronic exposure conditions.

The appropriate UF for these situations should be decided on a case-by-case basis until more definitive guidelines are available.

#### 4.3.9.2 Assignment of Confidence Levels

The selection of a $NOAEL_{[HEC]}$ or other appropriate measure of threshold response involves a process that incorporates scientific subjective judgment and statistical measures of significance. The qualitative and quantitative nature of this process results in an RfC associated with varying degrees of confidence that can be described as high, medium, and low.

A confidence level of high, medium, or low is assigned to the study used in the operational derivation, the overall data base, and to the RfC itself. Confidence ascribed to the RfC estimate is a function of both the confidence in the quality of the study and confidence in the completeness of the supporting data base together, with the data base

confidence taking precedence over that assigned to the study. High confidence in the RfC is an indication that the data base included investigation of a comprehensive array of noncancer toxicity endpoints, established from studies of chronic duration in various mammalian species, and that the study (or studies) established an unequivocal $NOAEL_{[HEC]}$. Therefore, a high confidence RfC is not likely to change as more data become available, with the exception of additional mechanistic data or sophisticated tests that may change the perspective of the evaluation. Low confidence in an RfC is usually applied to a derivation that is based on several extrapolations and indicates an estimate that may be especially vulnerable to change if additional data become available. If the individual study is of excellent quality, it most likely will receive a high confidence rating, although it may be subchronic in duration. Duration of the chosen study, as well as supporting studies and the spectrum of investigated endpoints (e.g., reproductive effects), are considered in the rating of confidence in the data base. Low confidence in the data base might be given to an excellent chosen subchronic study with few supporting studies and few endpoints examined. The confidence in the RfC then would reflect these two ratings by a rating of medium to low, indicating uncertainty (lack of confidence) and suggesting that further investigations may refine this number.

The level of confidence in a particular threshold value will be higher if it is derived from human data and supported by laboratory animal data. The parameters and factors involved in the evaluation of human data are described in Section 3.1.1. The degree of confidence in a particular laboratory animal study involves a number of parameters. These parameters include, but are not limited to, the following.

- Adequacy of study design
  - Is the route of exposure relevant to humans?
  - Were an appropriate number of animals and of both sexes used for determination of statistical significance?
  - Was the duration of exposure sufficient to allow results to be extrapolated to humans under different exposure conditions?
  - Were appropriate statistical techniques applied?
  - Were the analytical techniques sufficient to adequately measure the level of the test substance in the exposure protocol, including biological media?

- Is the animal species and strain appropriate as a surrogate for humans?

- Are the techniques for measurement of the biological endpoints scientifically sound and of sufficient sensitivity?

- To what degree may the biological endpoints be extrapolated (qualitatively or quantitatively) to humans?

- Demonstration of dose-response relationships

  - Were sufficient exposure levels used to demonstrate the highest NOAEL for the endpoint of concern?

  - Is the shape of the dose-response curve consistent with the known pharmacokinetics of the test substance?

  - Has the dose-response curve been replicated by or is it consistent with data from other laboratories and other laboratory animal species?

- Species differences

  - Are the metabolism and pharmacokinetics in the animal species similar to those for humans?

  - Is the species response consistent with that in other species?

  - Is the species from which the threshold value was derived the most sensitive species?

- Other factors

  - The number of biological endpoints evaluated and associated with dose-response relationships,

  - Sufficient description of exposure protocol, statistical tests, and results to make an evaluation, and

  - Condition of animals used in the study.

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# APPENDIX A

# ALTERNATIVE APPROACHES TO THE ESTIMATION OF NO-OBSERVED-ADVERSE-EFFECT LEVELS

The inhalation reference concentration (RfC) approach based on a lowest-observed-adverse-effect level/no-observed-adverse-effect level (LOAEL/NOAEL) paradigm is consistent with current methods for estimating human health risks from exposure to threshold-acting toxicants in water or food, such as those established by the Food and Drug Administration (Kokoski, 1976), the National Research Council (1977, 1980), the World Health Organization, the Food and Agricultural Organization (Bigwood, 1973; Vettorazzi, 1977, 1980; Lu, 1983), and other approaches used by U.S. Environmental Protection Agency (Federal Register, 1980; Stara et al., 1981; Barnes and Dourson, 1988). To date, these methods have generally considered only chronic or lifetime exposure to individual chemicals based on the assumption that "lifetime" data in laboratory animals are directly applicable to lifetime human exposures. As our understanding of the exposure-dose-response continuum is refined and the temporal aspects of the pathogenesis mechanisms elucidated (see Section 4.3.2), dose-response benchmark estimates for health risk characterization may be able to address intermediate duration, periodic, and other exposure scenarios with greater accuracy.

These methods generally estimate a single, constant daily dose that is low enough to be considered "safe" or "acceptable" (referred to as an acceptable daily intake [ADI]) or without appreciable risk (RfC). A number of scientific problems with this approach have been long recognized (Krewski et al., 1984; Crump, 1984; Brown and Erdreich, 1989). The first problem is that this method does not readily account for the number of animals used to determine the appropriate NOAEL. As described in Section 4.2 on designation of effect levels, the NOAELs or LOAELs that serve as the critical data in the RfC approach can be based on statistically significant or biologically significant increases in the frequency or severity of adverse toxic effects. For example, NOAELs have been defined for quantal endpoints that have nonzero background incidences by choosing an experimental exposure

level that does not contribute to a statistically significant increase in incidence of adverse effects when compared to a control group. Some NOAELs have been defined for continuous data by choosing an experimental exposure level that does not constitute a significantly different mean value for a parameter, indicating an adverse effect when compared to a mean value for a control group. Statistical significance, however, depends heavily on the design of the experiment, including sample size, the number of concentrations used, the spacing of the concentrations, and the arbitrary alpha level. Often the only information gained from the experiment used as the critical study is the presence or absence of statistical significance for an arbitrary alpha level at a small number of concentrations. Similarly, biological significance is often attributed to a concentration with little consideration of the impact of experimental design and no strict definition of the biological changes, suggesting that the designation of NOAEL or LOAEL is to an extent subjective. For example, if a chemical has a NOAEL based on 10 animals and another NOAEL with the same value but based on 100 animals, the risk assessor often will choose the NOAEL based on the larger study because it yields greater confidence in the resulting RfC. However, comparison of statistical power is not routinely done and the influence of sample size may not be taken into account when comparing disparate NOAELs. It has also been argued that the use of this approach encourages studies with smaller sample sizes, which reduce the power of the test. If these NOAELs were for different chemicals, similar RfCs might be derived, even though one would be associated with much less confidence.

The second problem with the current NOAEL/LOAEL approach is that the slope of the dose-response curve of the critical toxic effect is generally ignored in the estimation of the NOAEL. Many scientists have argued that this slope should in some way directly affect the estimate, with steep curves presumably yielding lower values because thresholds or greater toxicities are more quickly obtained with increasing concentration.

Furthermore, the current NOAEL/LOAEL approach to noncancer dose-response assessment yields an RfC estimate that is presented as a single number. As such, it reflects neither the statistical variability in the NOAEL resulting from study design factors nor the inherent variability for which uncertainty factors are applied to extrapolate from the data base to the RfC. The result of this variability is the unknown range of uncertainty in the estimate. Exposure estimates to which the dose-response estimate must be compared are also associated

with a range of uncertainty and many exposure models now express explicitly this variability as a distribution. Risk management decisions for regulation or enforcement need more quantitative information on the inherent and recognized uncertainties in this assessment.

This appendix defines and illustrates alternative approaches to derivation of estimates that could be used as analogues to the NOAEL. Many of these approaches offer solutions to some of the criticisms of the NOAEL/LOAEL approach outlined above and these attributes will be highlighted. Even so, no method is without inherent problems. Guidance is under development that describes the application of "benchmark" concentration-response modeling (Section A.2) to derive dose-response estimates such as the inhalation RfC. Recently, EPA and the Risk Science Institute of the International Life Sciences Institute (ILSI) sponsored a workshop entitled, "Workshop on Benchmark Dose Methodology". A summary paper from the deliberations at these meetings discusses definitions and criteria for the use of a benchmark approach to estimate a reference dose or reference concentration (Barnes et al., 1994). The Risk Assessment Forum is also working on guidance that is anticipated to be published as a "purple book". The reader is referred to these additional sources and is encouraged to appreciate that development of guidance awaits consensus on issues raised both herein and in these additional materials.

It is worthwhile to emphasize, as it will be noted in subsequent sections of this appendix, that the toxicological decision as to what constitutes adversity (i.e., the decision that a specified effect is adverse and what the associated severity is), particularly across different endpoints, remains perhaps the most sensitive parameter in any of these procedures regardless of the mathematical model applied. Using quantal data, for example, it is a decision based on toxicological judgment that determines whether 10% or 30% incidence of a given lesion should be a concern. Similarly, toxicological or clinical insight may be required to determine if a particular change in a continuous parameter (e.g., pulmonary function decrement) is adverse relative to a normal population value or between a control and an exposed cohort. To date, there has not been adequate appreciation by toxicologists and biostatisticians alike of the interdependence of the decision to designate an effect as biologically significant and the decision to estimate a response at a given level from the mathematical model. Perhaps awareness of the interdependence is the single most important factor that requires systematic development before any of these approaches can be

implemented consistently. The decision on the definition of adversity or biological significance is termed designation of the "specified health effect" for purposes of discussion in this appendix. The concept of a specified health effect is not new and is related to the concept of "relative potency" (Jarabek and Hasselblad, 1991). Finney (1978) defines a relative potency in his description of a direct assay. A direct assay is one in which "...doses of the standard and test preparations sufficient to produce a specified response are directly measured. The ratio between these doses estimates the potency of the test preparation relative to the standard...." Note that the choice of a "specified response" is key to the definition.

Because all of the approaches presented herein have not yet been applied routinely to the types of data generally encountered when evaluating the health effects information available on the majority of inhaled chemicals, aspects that require further development and consideration in order to use these alternatives will also be presented.

## A.1 NO-STATISTICAL-SIGNIFICANCE OF TREND (NOSTASOT)

A statistically more accurate approach than the traditional NOAEL/LOAEL for estimating a NOAEL when several exposure levels are available is the "no statistical significance of trend" (NOSTASOT) approach proposed by Tukey et al. (1985). The underlying principle is to sequentially test for a linear trend until significance is no longer reached. As described by Tukey et al. (1985), the procedure is applied to all of the data first and then entails sequentially deleting the highest exposure groups in succession downward (i.e., "top-down" analysis). In this manner, the highest exposure level at which the response is not significantly different from controls is determined to be the NOSTASOT, which could therefore be considered a NOAEL.

### A.1.1 Approach Advantages

The advantage of the NOSTASOT approach is that it offers a simple yet fairly robust method to determine a NOAEL by testing for a trend in all exposure levels (including controls). As such, it utilizes more of the concentration-response information than individual comparisons of exposed and control groups.

## A.1.2 Application Issues and Development Needs

As proposed by Tukey et al. (1985), the NOSTASOT procedure tests for a statistical significant trend in a series of exposure levels (including controls) until the highest level at which the trend is nonsignificant is reached. This highest level is defined as the NOSTASOT exposure and would be used as a surrogate to the NOAEL in derivation of an RfC. The last concentration at which statistical significance was achieved would be a LOAEL. The method was developed for application to data from experiments involving multiple groups of animals of approximately the same size at different dose/exposure levels, including a zero-dose control. In such cases, the NOSTASOT method may be preferred because it includes more information and may have greater statistical power than multiple comparisons of different experimental groups to a control group. However, despite its robustness, the NOSTASOT approach remains sensitive to dose spacing. It is also sensitive to sample size when applied to grouped data. Application to epidemiologic data with individual exposure data or to continuous response measures is not straightforward.

An alternative to the NOSTASOT approach is to start at the lowest noncontrol exposure or dose level and move upward (i.e., "bottom-up" analysis). The objective is to determine the highest level of nonsignificance before a significant difference is detected. The highest nonsignificant dose would be declared the NOAEL and the first statistically significant dose a LOAEL in this analysis. The sensitivity to sample size and dose spacing of the NOSTASOT approach is illustrated by the difference between a "top-down" versus "bottom-up" analysis. In most animal experiments for which the procedure was developed, with groups of the same size exposed to typically only at one, two, three, or four levels, the NOSTASOT would be the same whether analyzed from the top down or the bottom up, assuming that the response is monotonic. However, in data sets with a large number of exposure levels or with individual exposure data, the top-down and bottom-up analyses may yield very different estimates (i.e., a LOAEL from the bottom-up analysis may be below a NOAEL from the top-down analysis). This is conceivable with some nonlinear and/or nonmonotonic data sets (Davis and Svendsgaard, 1990). It is therefore necessary to apply the method in a manner that recognizes possible nonlinearities in the data (e.g., due to a sensitive subpopulation responding at low concentrations). Such complications warrant consideration when applying the NOSTASOT approach.

## A.2 "BENCHMARK" CONCENTRATION-RESPONSE MODELING

Concentration-response modeling, recently referred to as the "benchmark dose" approach, has been proposed as an improvement on the NOAEL/LOAEL approach (Crump, 1984). The "benchmark" approach, as defined in this discussion, is the use of a specific mathematical model (e.g., Weibull, logistic, polynomial) to determine a concentration (applied dose) and its lower confidence bound that is associated with a predefined effect measure (e.g., 10% response of a dichotomous outcome) as the "benchmark". Application of this approach (Kimmel and Gaylor, 1988) has been proposed for developmental endpoints, which are generally dichotomous (quantal) in nature, but it has yet to be applied widely to other noncancer outcomes.

Figure A-1 illustrates the benchmark approach as applied to laboratory animal developmental data. A mathematical model (e.g., Weibull, logistic, polynomial) is applied (fitted) to the experimental effects data to estimate a maximum likelihood estimate (MLE) or concentration-response function. The 95% confidence limit is calculated using information on sample size and variance. It has been recommended that limits based upon the distribution of the likelihood ratio statistic be used as the method of choice for this calculation (Crump, 1984). The possible analogues to a NOAEL can then be estimated. For example, the 10th percentile of an effect level could be designated as synonymous to "no adversity" and the concentration corresponding to the MLE of that effect level used as the "effective concentration" ($EC_{10}$). The lower confidence bound on the $EC_{10}$ could also be used and is shown as "$LEC_{10}$". A linear interpolation has also been proposed (Gaylor and Kodell, 1980; Kimmel and Gaylor, 1988) that allows estimation of upper limits on risk for convex dose-response curves. For example, as shown in Figure A-1, at a dose of $LEC_{10}$ divided by an uncertainty factor (UF), the "true" unknown risk in the low-dose regions is expected to be less than that associated with the linear extrapolation if the "true" dose-response curves upward.

### A.2.1 Approach Advantages

Compared to the NOAEL/LOAEL approach, benchmark concentration-response modeling has the advantages that it utilizes more information from the dose-response curve, is less influenced by experimental design (e.g., exposure level spacing), and is sensitive to the

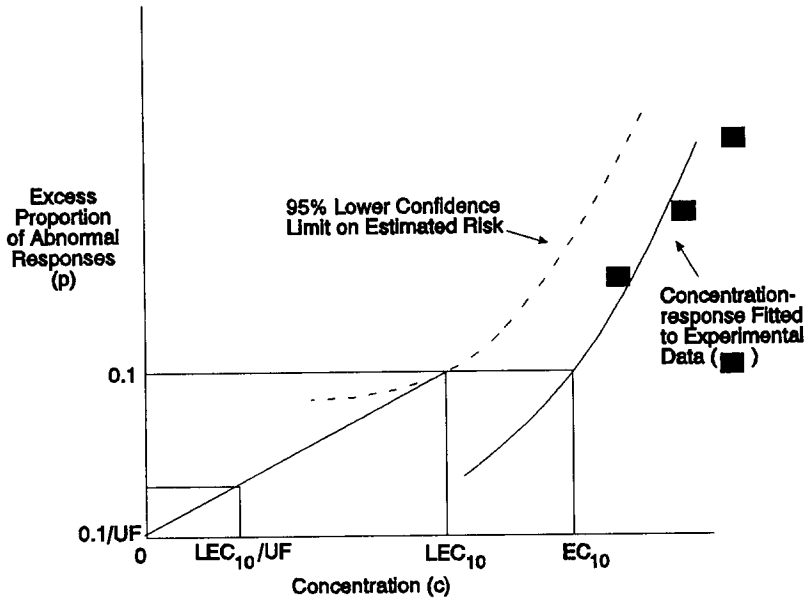


**Figure A-1. Graphical illustration of proposed low-dose risk estimation for the proportion of abnormal responses in developmental toxicity.**

Adapted from Kimmel and Gaylor (1988).

influence of sample size. It is important to note that this approach is sensitive to sample size only when the "benchmark" is defined as the lower confidence bound. The MLE alone is not influenced by sample size.

## A.2.2 Application Issues and Development Needs

Application of this approach to the myriad of endpoints that can constitute noncancer toxicity will require significantly greater effort directed at modeling continuous data. A limitation may be finding data sets appropriate for modeling. Guidance must be developed on choice of model structures and on goodness-of-fit criteria for models, especially whether or not it is appropriate to superimpose model structures on data that only have one dose group associated with a nonzero response (relative to control or background). Whether or not there is a biological basis (e.g, for certain endpoints) for selecting certain model structures also warrants investigation.

Use of the benchmark approach still requires dosimetric adjustment to a human equivalent concentration (Section 4.3) and for application of UFs to account for extrapolations (Section 4.3.8.1). Dosimetric adjustment to account for interspecies differences should be applied before the data are modeled.

Application of UFs in a fashion analogous to that used with the NOAEL/LOAEL paradigm have been proposed for use with the benchmark approach (Dourson et al., 1985, 1986). That is, a benchmark estimate for a more severe endpoint (e.g., liver necrosis), essentially equivalent to a LOAEL, would warrant application of an additional UF, whereas the endpoint judged as less severe (e.g., slight body weight decrease) would not. Application of UF for intraspecies variability, subchronic duration, and data base may also be appropriate.

Another approach for the application of UFs for dichotomous data has been proposed using the linear interpolation from the $LEC_{10}$ through the origin as shown in Figure A-1 (Kimmel and Gaylor, 1988). As shown on Figure A-1, if UF represents an uncertainty factor, then the true unknown risk at an exposure concentration of $LEC_{10}/UF$ is expected to be less than 0.1/UF. This procedure is conservative with respect to risk when the dose-response is convex (curving upward). Therefore, an advantage is that an upper limit on the risk is estimated. The size of the factor depends on the desired level of risk. For example, a factor of 10 applied to the $LEC_{10}$ would result in a risk less than $10^{-2}$. This approach assumes that the incidence in humans on which the "acceptable risk" decision is based is equivalent to the observed incidence of a given lesion in the experimental animals.
An equivalent procedure for continous data would necessitate an assumption that the mean severity or magnitude of the observed effect in the exposed population relative to the control (or relative to a normal reference) was equivalent in experimental animals and humans.

These UF approaches essentially result in subthreshold estimates, similar in intent to the RfC, provided the $LEC_{10}$ is considered to be analogous to a NOAEL and if the designation of the specified health effect is unequivocal. However, the designation of a specified health effect is a question of both biological and statistical significance. Various levels (e.g., $EC_{01}$, $EC_{05}$, and $EC_{10}$) have been proposed that could be considered as a NOAEL criterion

(Gaylor, 1983; Kimmel and Gaylor, 1988; Fabro et al., 1982).[1] If one incidence level were to be designated as the NOAEL criterion (e.g., 10%), a dose-response estimate could be based on either 10% nasal hyperplasia or 10% proximal tubule necrosis, unless the severity of the endpoint is taken into account. Intimate knowledge of the spectrum of severity within a pathogenesis continuum for an individual endpoint may be required before criteria can be established for designating specified health effects. Further, in order to compare across the various endpoints associated with noncancer toxicity, it may be necessary to "normalize" (e.g., designate the 50th percentile as the criterion for a minimally adverse effect and the 1st percentile for a severe effect), but this would require consensus on definitions of severity. The interaction with model structure may also be influenced by these criteria. For example, model "fit" and variability in the resultant estimate would be different for lesions designated at the $EC_{50}$ and the $EC_{01}$ and determined at the associated lower confidence bound. The relationship of these different estimates to applied UFs would also be different. The choice of the mathematical model structure generally makes relatively little difference down to approximately the 1% risk level. Estimation of excess risk above background in the region below that level can become more dependent on the choice of model structure than on the true dose-response curve. Although previous use of the "benchmark" approach avoided this controversy because developmental endpoints do not distinguish degrees of severity to a large extent, such issues are critical for development of this approach as an application to all the other common noncancer endpoints.

Derivation of a dose-response estimate by the benchmark approach also does not preclude evaluation of the data base for completeness. A comprehensive array of endpoints must be evaluated to identify potential hazard for various target tissues regardless of the way individual endpoints may be modeled. Once the individual specified health effects are decided, determinations of the appropriate species and critical effect representative of the threshold for the overall data array must be evaluated as described for the RfC methodology in Section 4.3.7.

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[1]It could also be argued that the exposure estimated to be the 5$^{th}$ percentile is really a lower confidence limit of the exposure causing a specified effect. In that sense, any point below it is associated with no effect, and therefore the 5$^{th}$ percentile (or any other lower tail percentile) could be considered as a NOAEL. Designation criteria for the LOAEL, however, will be problematic as outlined above.

## A.3 APPLICATION OF BAYESIAN STATISTICS

As discussed in Section 4.3.7, the analysis of noncancer toxicity data often involves evaluation of data and a synthesis of information together in order to determine a representative level for the threshold region of the data array. For example, sometimes a NOAEL from one study may be used in conjunction with a NOAEL from another. Data from a "free-standing" NOAEL are often used in a qualitative sense but cannot be used in a dose-response model. The advantage to such a synthesis is the utilization of more information rather than the reduction of data to a single study and its effect level, a practice that is recognized as a significant limitation to the RfC and benchmark approaches described above.

A Bayesian statistical approach has been proposed that both statistically incorporates the attributes of the benchmark approach (incorporates influence of sample size and shape of the dose-response curve) as well as offers the advantages of (1) visual display and description of the uncertainty in the risk estimates, (2) allows for explicit synthesis of dose-response estimates together when determined appropriate, and (3) allows for explicit incorporation of uncertainty in the exposure characterization (Jarabek and Hasselblad, 1991; Hasselblad and Jarabek, 1994).

The general approach proposed has been published under the title of the Confidence Profile Method (Eddy et al., 1992). It combines the standard classical and Bayesian statistical methods to produce likelihood functions and posterior distributions for parameters of interest. Although the likelihood functions and posterior distributions have very different interpretations, their shape is usually extremely similar. The likelihood function can be used to compute confidence intervals. The posterior distribution is a continuous plot describing belief about the location of the parameter of interest (i.e., for dose-response estimation purposes, about the dose associated with a specified health effect). The basic formula of Bayesian statistics is

$$p'(\theta) = L(\theta \mid \text{data})\ p(\theta), \qquad \text{(A-1)}$$

where:

$\theta$ = parameter of interest,
$p(\theta)$ = prior distribution for $\theta$,
$L(\theta \mid \text{data})$ = likelihood for $\theta$ given new data, and
$p'(\theta)$ = posterior distribution for $\theta$. Because $p'(\theta)$ will become the prior for the next experiment, it is denoted by the same letter.

Consider the following simple example of a continuous effect measure. Assume that the health effect, y, is related to the exposure, x, given by the model

$$y = \beta x. \tag{A-2}$$

Assume further that we wish to specify a particular health effect, $y_0$, and then estimate the exposure corresponding to this effect as

$$\theta = y_0/\beta. \tag{A-3}$$

Because $\theta$ is not defined for $\beta \leq 0$, it is reasonable to choose the prior for $\theta$ as $p(\beta) = 1$ for $\beta > 0$ and $p(\beta) = 0$ elsewhere. This corresponds to the belief that exposure to a toxic chemical is not beneficial. The prior just described is the horizontal dashed line in Figure A-2. Assume that an experiment to determine information about $\beta$ was conducted, resulting in the likelihood, $L(\beta)$, shown as a dotted line in Figure A-2. Note that this likelihood is positive for values of $\beta$ less than 0. The posterior distribution, $p'(\beta)$, is the product of the likelihood function and the prior (properly normalized to be a probability distribution) and is shown as a solid line in Figure A-2. Note that this distribution has the same general shape as the likelihood function, except that it has no mass below zero. This kind of distribution is often referred to as a truncated distribution. The posterior distribution of $\theta$ can be calculated from the posterior distribution of $\beta$. It should be emphasized that the mathematical modeling of these data was not different for these effect measures than that which could be achieved using a benchmark approach, but the expression as a normalized posterior distribution is the difference that provides for visual inspection and statistical combination of data. The posterior distribution, $p'(\beta)$, can be used as a prior if another experiment is conducted giving additional information about $\beta$, and the application of Bayes'

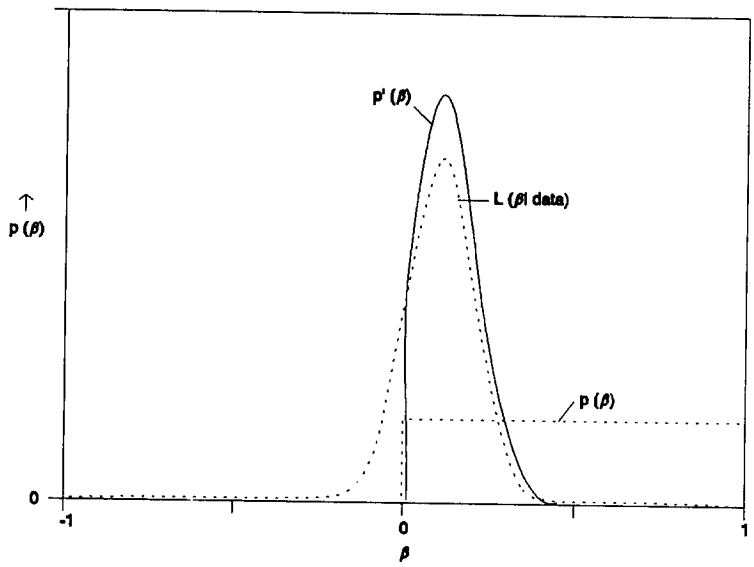


**Figure A-2. Schematic of computing a posterior distribution [p′ (β^C)] from a likelihood function [L (β | data)] and a prior distribution [p (β)].**

Source: Jarabek and Hasselblad (1991).

formula repeated. (Note: In the following applications, $\theta$ [the parameter of interest] is designated as $x_0$, the exposure concentration associated with a specified health effect.)

The proposed Bayesian approach is illustrated in Figures A-3 through A-5. Figure A-3 shows the dose-response from a logistic model superimposed on the dichotomous data for nasal turbinate lesions in mice exposed to *n*-hexane (data of Dunnick et al., 1989). For illustration purposes, an incidence of 10% (shown by the dashed line) is designated as the specified health effect (Jarabek and Hasselblad, 1991). Figure A-4 shows the posterior distribution for the *n*-hexane concentration associated with that 10% incidence. Figure A-5 shows the statistical synthesis together of posterior distributions of two different concentrations associated with specified health effects (one respiratory, the other neurotoxicity) of two studies (Dunnick et al., 1989; Sanagi et al., 1980.) The results of this synthesis were in general agreement with the NOAEL used for the RfC derivation for this chemical (IRIS, 1990) and with the benchmark approach for either of the two studies (data not shown). Although experimental details are provided elsewhere (Jarabek and Hasselblad, 1991), the two data sets represent both continuous and dichotomous effect measures, illustrating the ability of the Bayesian approach to address different outcomes.

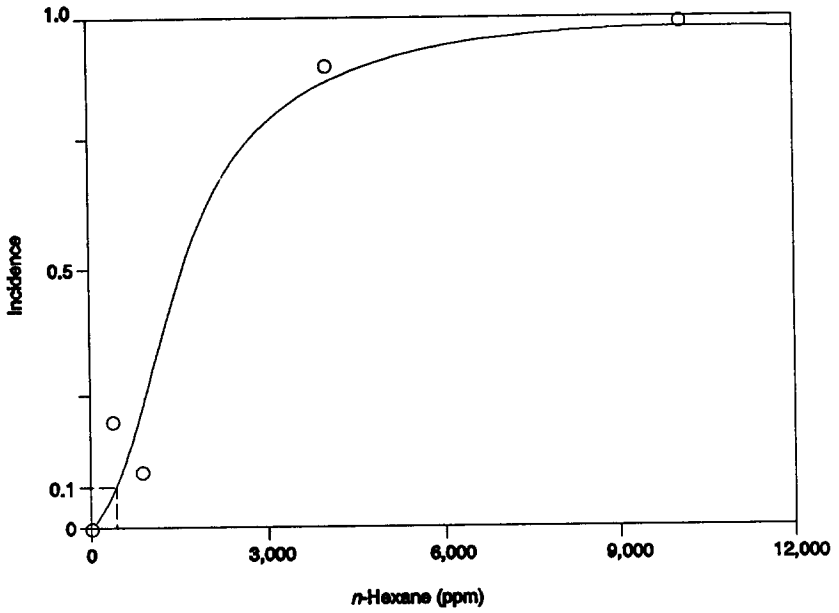


**Figure A-3. Incidence of nasal turbinate lesions in B6C3F1 female mice exposed to *n*-hexane for 13 weeks. Data of Dunnick et al. (1989).**

Source: Jarabek and Hasselblad (1991).

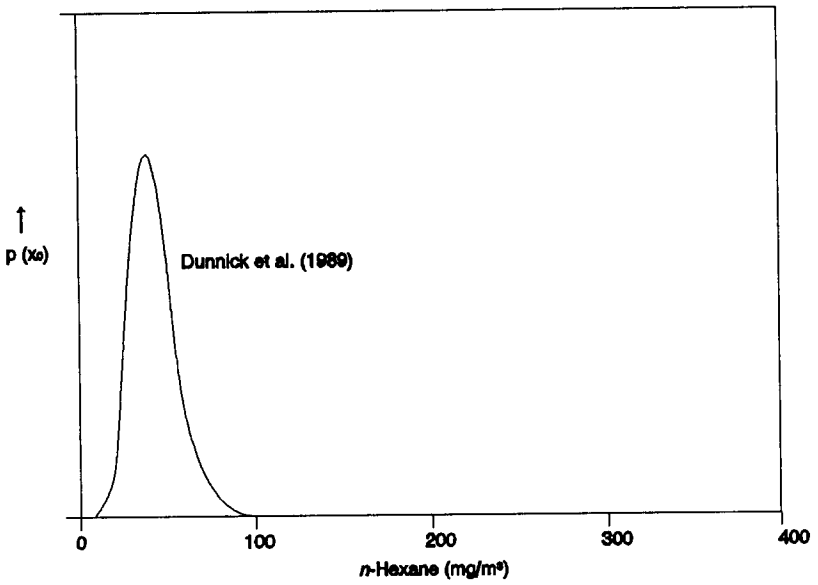


**Figure A-4. Posterior distribution for the *n*-hexane concentration associated with the specified health effect in Figure A-3. Data of Dunnick et al. (1989).**

Source: Jarabek and Hasselblad (1991).

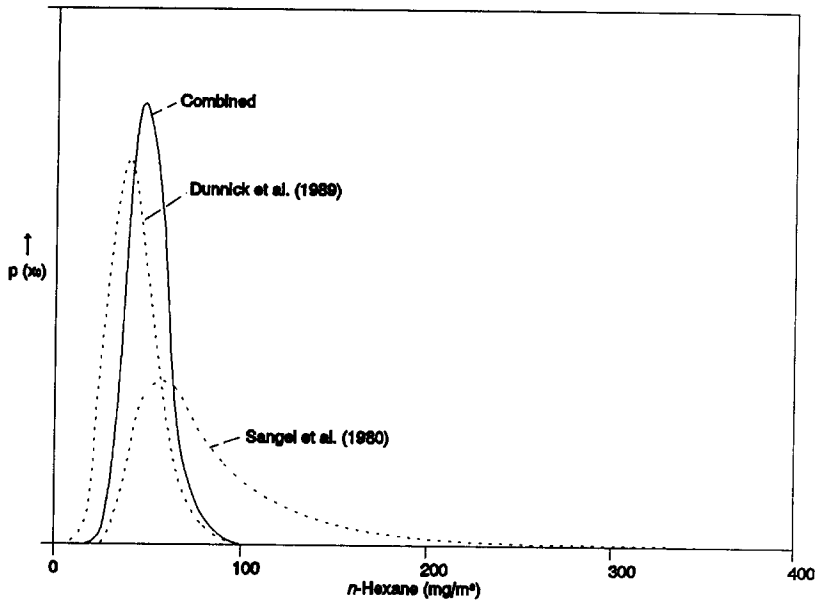


**Figure A-5. Posterior distribution for the concentration of *n*-hexane associated with the specified health effects from the combined evidence of Sanagi et al. (1980) and Dunnick et al. (1989).**

Source: Jarabek and Hasselblad (1991).

## A.3.1 Approach Advantages

Visual presentation of data is a powerful tool for analysis and communication (Cleveland, 1985). Visual inspection of the posterior distribution concurs with the variability of the data and provides much information about the usefulness of the health effects data for dose-response evaluation. The shape of the posterior distribution for the data of Dunnick et al. (1989), in contrast to that of Sanagi et al. (1980), easily highlights that these data were generated from an investigation with an adequate number of animals and test concentrations with a resultant tighter distribution and reduced variance. The skewed posterior distribution for the data of Sanagi et al. (1980) results from its greater variance and small sample size. The value of visual presentation is again illustrated in Figure A-6. This figure shows the posterior distributions for the concentration of manganese (Mn) associated with specified health effects (all approximately the same measure of neurotoxicity) from three different studies. The visual presentation of the posterior distribution easily communicates that the data of Chandra et al. (1981) were highly variable and in fact do not add much information

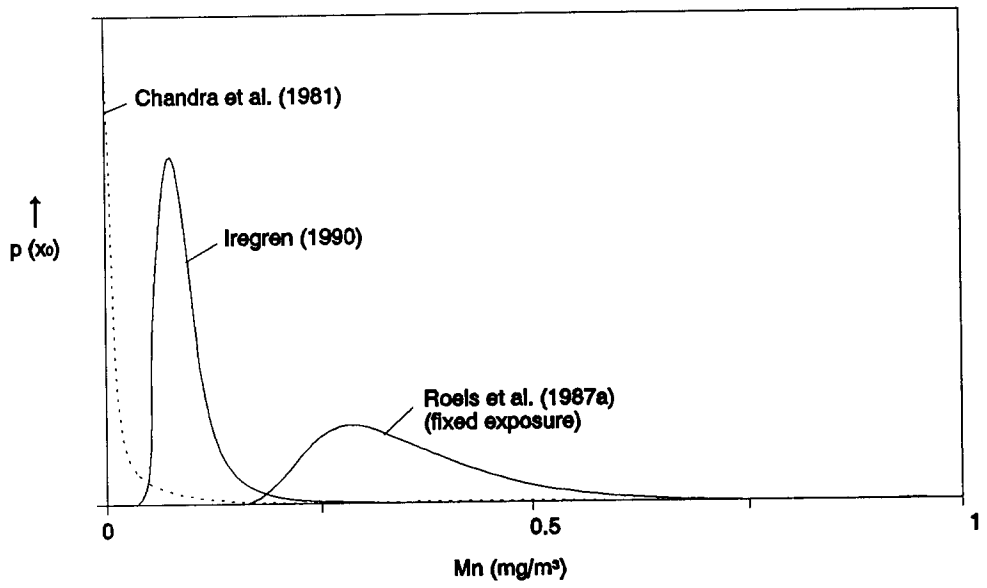


**Figure A-6. Posterior distributions for the manganese (Mn) concentration associated with specified health effects from each of three studies: Roels et al. (1987a), Iregren (1990), and Chandra et al. (1981).**

to the synthesis. An appreciation of this variability would not have been imparted from the numeric reporting of the estimate alone. Even if the percentile values were reported and some sort of analysis on the spread is done (e.g., compare the ratio of the 95th to 50th percentile for all studies), the communication of the reliability of these data is not as straightforward as that of the visual display.

The Bayesian approach also allows for explicit incorporation of uncertainty in the exposure estimates of the studies being evaluated. The influence that direct application of uncertainty in the exposure estimate can have on the resultant dose-response estimate is illustrated in Figures A-7 through A-9. These figures illustrate the influence of variability in exposure characterization for the health effect data used to determine the dose-response. Because the posterior distribution now expresses the dose-response estimate as a distribution instead of a point estimate, this approach allows the dose-response distribution to be combined statistically with an exposure distribution for risk characterization. Therefore, such

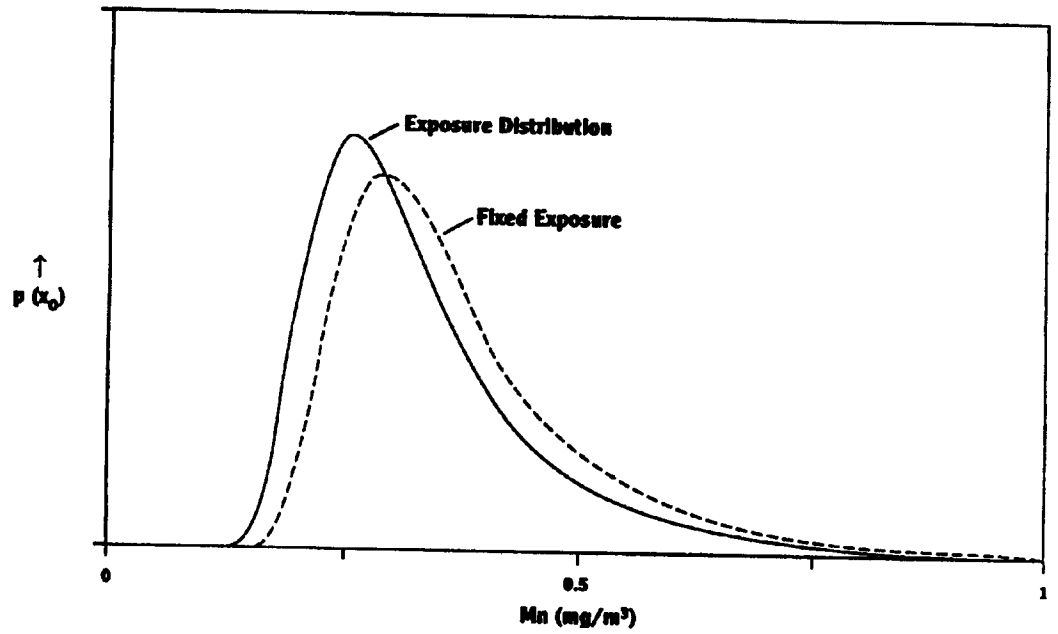


**Figure A-7.** Posterior distribution for the manganese (Mn) concentration associated with specified health effect using either exposure or estimated exposure distribution. Data Roels et al. (1987a,b).

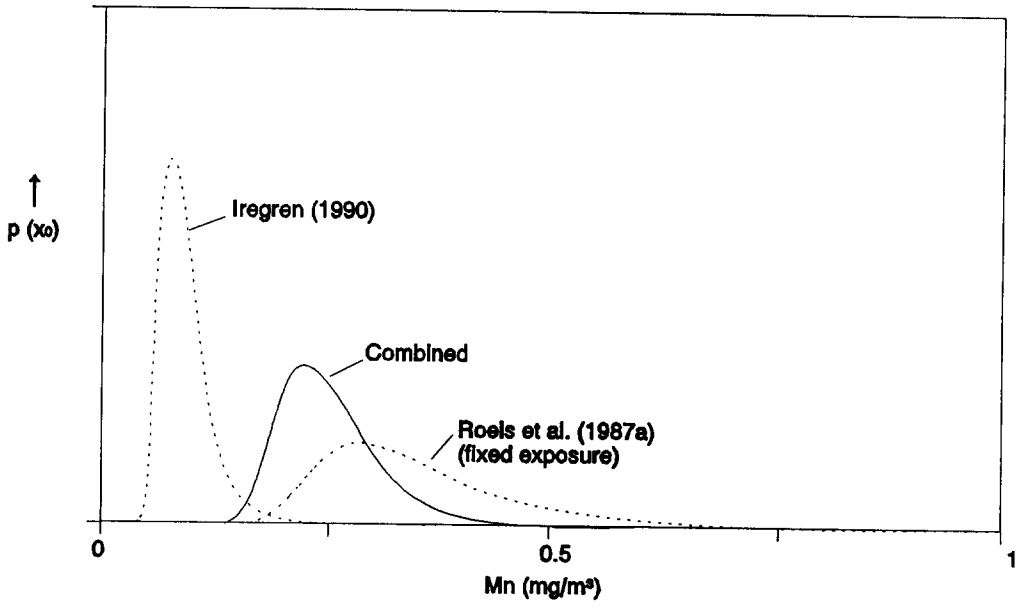


**Figure A-8.** Posterior distribution for the concentration of manganese (Mn) associated with specified health effect from the combined evidence of Iregren (1990) and Roels et al. (1987a) with fixed exposure.

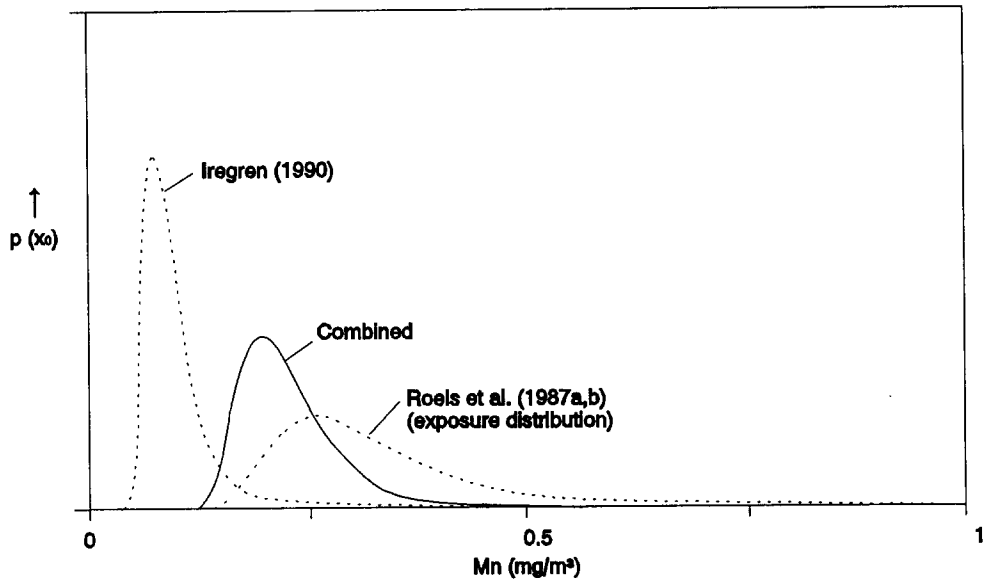


**Figure A-9. Posterior distribution for the concentration of manganese (Mn) associated with specified health effect from the combined evidence of Iregren (1990) and Roels et al. (1987a,b) with exposure distribution.**

presentation will allow explicit incorporation of uncertainty in the dose-response and exposure estimate to be carried through to the risk characterization step and could provide more information on which to base management decisions.

The Bayesian approach offers the advantages of the benchmark approach in that it takes into account the influence of sample size and shape of the dose-response curve. However, it is the only currently viable approach that offers the ability to statistically combine evidence from different investigations. Such synthesis is routinely done with data without explicit statistical handling of experimental design.

The Bayesian approach offers the advantages of the benchmark approach in that it takes into account the influence of sample size and shape of the dose-response curve. However, it is the only approach that offers the ability to statistically combine evidence from different investigations. Such synthesis is routinely done with data without explicit statistical handling of experimental design.

### A.3.2 Application Issues and Development Needs

As mentioned, the Bayesian approach is essentially the same method as the benchmark approach up until the expression of the posterior distribution. Therefore, most of the issues under Section A.1.2 apply to the development of this approach as well.

In addition, application of the statistical synthesis capability of the approach will require guidance development as well. Figure A-3 presents the statistical combination of data with different endpoints: neurotoxicity (Sanagi et al., 1980) and respiratory tract effects (Dunnick et al., 1989). The resultant posterior distribution for the combined evidence of different endpoints was not drastically different relative to the individual distributions from which it was derived. This may be due to the fact that both studies investigated very sensitive endpoints (i.e., near the threshold or subthreshold region). Perhaps when data are not comparable with respect to assayed endpoints, but the data represent very sensitive endpoints, then the combination of these data provide a more likely estimate of the concentration of concern. The data combined for Mn on the other hand, were all for the same specified health effect (neurotoxicity). The exclusion of the Chandra et al. (1981) data was on the basis of statistical considerations. Future development of this approach will have to develop guidance on limitations for data combination both for statistical and biologically motivated concerns.

## A.4 CATEGORICAL REGRESSION: USE OF DOSE-GRADED DATA

Not all data are expressed as quantal or continuous data that are readily amenable to available standard dose-response models. Results are often reported as "categorical" (i.e., descriptive or severity-graded results [e.g., a particular dose group exhibited "mild" toxicity]). As mentioned in the advantages for the Bayesian approach, other studies that are not explicitly designed to examine dose-response relationships, such as single-dose studies or mechanistic studies, may nonetheless provide useful data that should be incorporated into the data array analysis.

An analysis method that allows the combination of quantal data with categorical data and models the relationship between the severity of the effect against the exposure concentration and duration has been proposed for chronic oral toxicological data

(Hertzberg, 1989). Guth et al. (1991, 1993) have extended this work to inhalation exposures and have proposed a regression analysis method that provides for incorporation of both quantal and dose-graded data and for data across different durations. The method has been proposed in order to utilize as much of the available data as possible for the evaluation of short-term inhalation exposures defined as less than or equal to 24 h in duration.

A categorization scheme is used for the quantitative exposure-severity analysis, with severity category as the dependent variable and with concentration and exposure duration as independent variables. The severity scheme consists of three categories representing NOAELs, adverse effect levels (AELs), and lethality. More complicated severity-ranking schemes can be applied but become contentious due to the difficulty in equating severity of effect measures across target organs, endpoints, and species (Guth et al., 1991; 1993).

The form of the model for regression analysis is

$$LN(p/1 - p) = A_i + B_1 LN(\text{Concentration}) + B_2(\text{Duration}), \qquad \text{(A-4)}$$

where p is the probability that, at a given concentration and duration of exposure, severity will be less than or equal to the severity category with rank = i, and A and B are estimated model parameters. The model is solved for $P = 1 - p$, or the probability that, at a given concentration and duration, the severity will be greater than the severity category with rank = i. The regression analysis assumes constant slope parameters, hence the values of $B_1$ and $B_2$ are constant across severity categories. The order or rank of the categories is used, rather than the numerical values.

The model output is readily interpreted in the context of risk assessment. Figure A-10 illustrates the method applied to categorical data for exposures of less than 8 h in duration and shown as NOAELs, AELs, or lethality. Although longer exposure regimens are appropriate as an alternate method to derive a NOAEL for the RfC, this example based on acute data is offered. The maximum likelihood model fit is shown by the line representing the model prediction of $p = 0.1$ that severity is greater than the NOAEL category (i.e., that the predicted effect would be in the "adverse" range or higher) at the corresponding exposure concentration and duration.

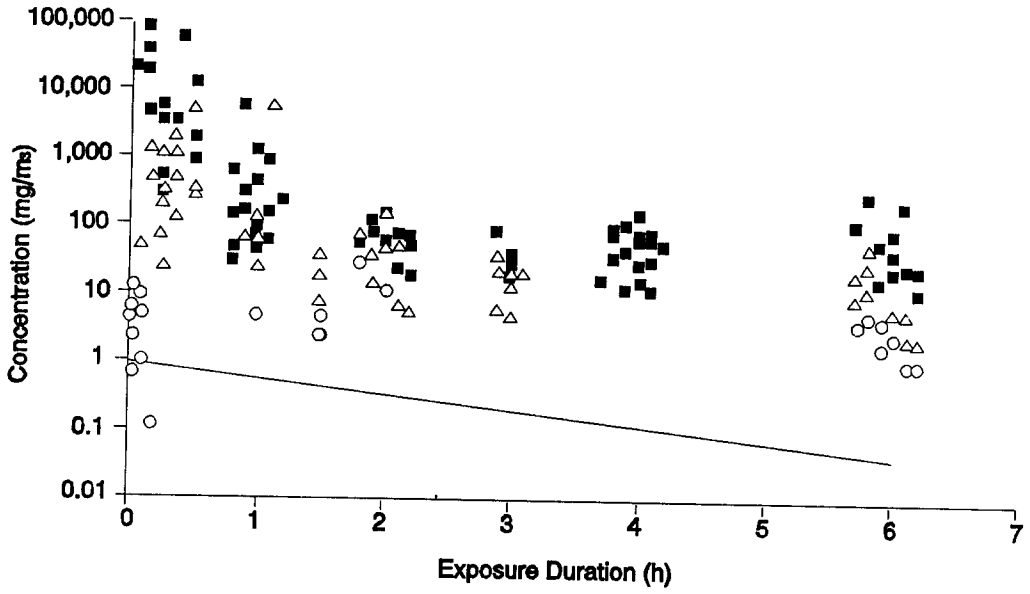


**Figure A-10. Categorical data from published results on methyl isocyanante for exposures of less than 8 h in duration and shown as NOAEL (circles), AEL (triangles), or lethality (squares). The maximum likelihood model fit is shown by the line representing the model prediction (p = 0.1) that severity is greater than the NOAEL category at the corresponding exposure concentration and duration.**

Source: Guth et al. (1993).

## A.4.1 Approach Advantages

Health risk assessments generally require evaluation of several types of toxicity data derived from several different species, different doses, different exposure durations, varied endpoints, and varied quality. This variety often makes the health risk assessment extremely difficult. Therefore, it is valuable to have all such toxicity data displayed simultaneously and this approach offers the advantage of a graphic presentation. Exposure-duration response trends, if present, are clearly delineated. This insight may provide a possible strategy for disaggregation of data according to a duration window and/or for a particular endpoint.

This categorical analysis approach also offers the advantages of allowing the use of data that is not otherwise amenable to quantitative concentration-response analysis, such as categorical data and data from single-dose studies, and of incorporating both concentration and duration of exposure as explanatory variables. Various types of data (dichotomous,

continuous, categorical) can be considered simultaneously by converting each to a categorical descriptor. The estimates from this approach applied to short-term data have been shown to be in general agreement with estimates obtained from both the benchmark and NOAEL/LOAEL approaches (Guth et al., 1991; Guth et al., 1993).

The approach also offers the advantage of providing estimates for a range of exposure durations. Interpolation along this NOAEL boundary can be performed to estimate the NOAEL for any desired partial-lifetime exposure, rather than a linear prorate of the point estimate value at one given duration as is currently done with many approaches. It should be noted again that although the data shown here are truncated to exposures of less than 24-h duration, data can be incorporated for any duration and have been applied to the entire data sets on chemicals, regardless of duration (Dourson et al., 1986).

## A.4.2 Application Issues and Development

Development of this approach requires guidance on model application, particularly minimum data base requirements. For example, if data are too sparse or when the effect levels are far apart, often the model will not converge. Figure A-11 shows the model fit to the same data as in Figure A-10 but with the exclusion of lethality data. The presence of the lethality data influences how the model addresses the boundary line between "adverse" and "no-adverse" levels. It is also a question as to whether lethality data are appropriate to use for dose-response assessment that intends to be protective of public health. When the data are on one type of specified health effect (e.g., 2% carboxyhemoglobin in blood) in a single species (humans), the model shows remarkable agreement with estimates generated by a PBPK model for the same specified effect (Figure A-12). When an array of different endpoints are available from a number of different species, as shown in Figure A-13, then the choice of an endpoint may not be as straightforward. Therefore, the biological rationale for model application also needs to be refined, especially on whether to aggregate or disaggregate data on individual endpoints. If disaggregation results in convergence failure, then it could be argued that this approach using all the available data provides a conservative estimate of a NOAEL boundary and may be more certain than one derived from a single study. One approach to disaggregation of data may be based on respiratory versus extrarespiratory effects (and perhaps segregation of extrarespiratory endpoints) because it is likely that different

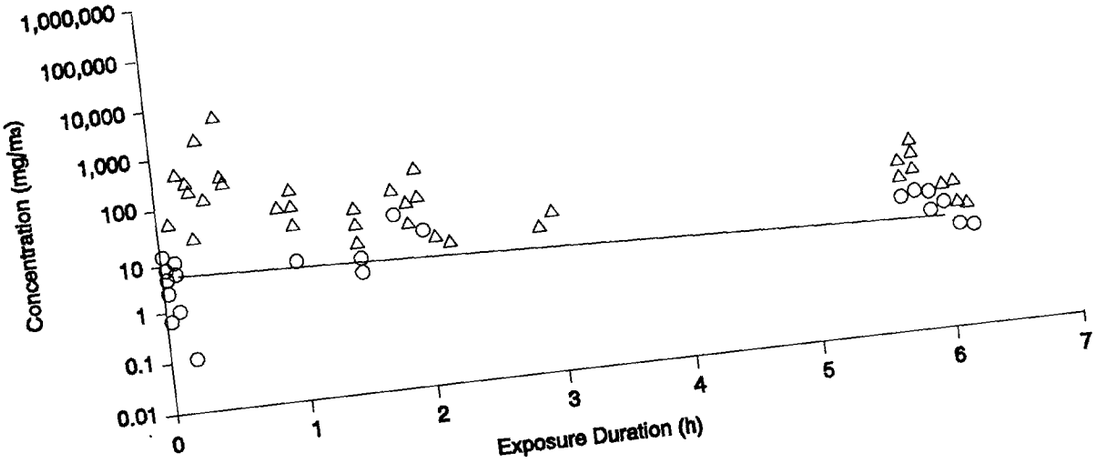


**Figure A-11. Categorical data from published results as in Figure A-10, excluding lethality data.**

Source: Guth et al. (1993).

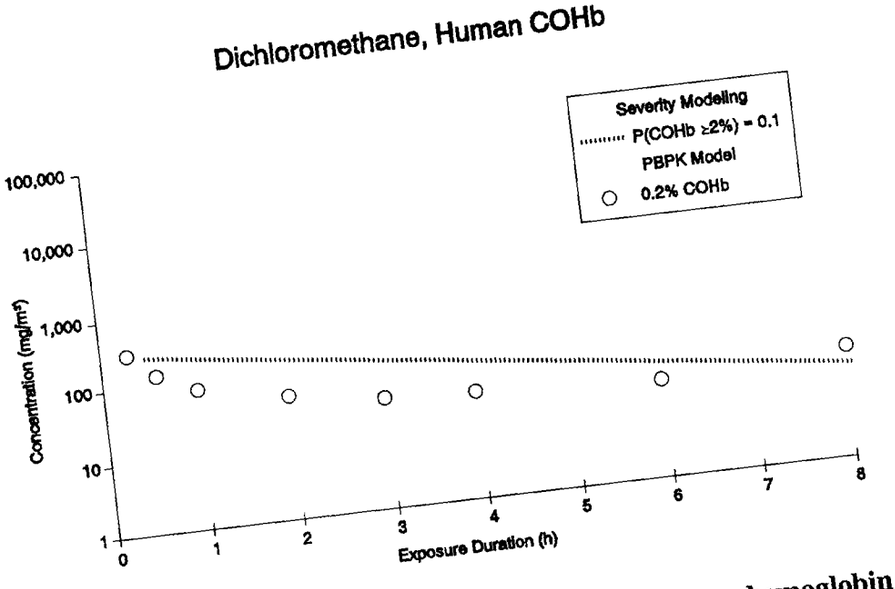


**Figure A-12. Categorical regression analysis for data on carboxyhemoglobin (CoHb) in humans. Specified adverse health effect was 0.2% CoHb. Circles indicate PBPK model estimates (Andersen et al., 1991) to achieve same 0.2% CoHb at various concentration and duration combinations.**

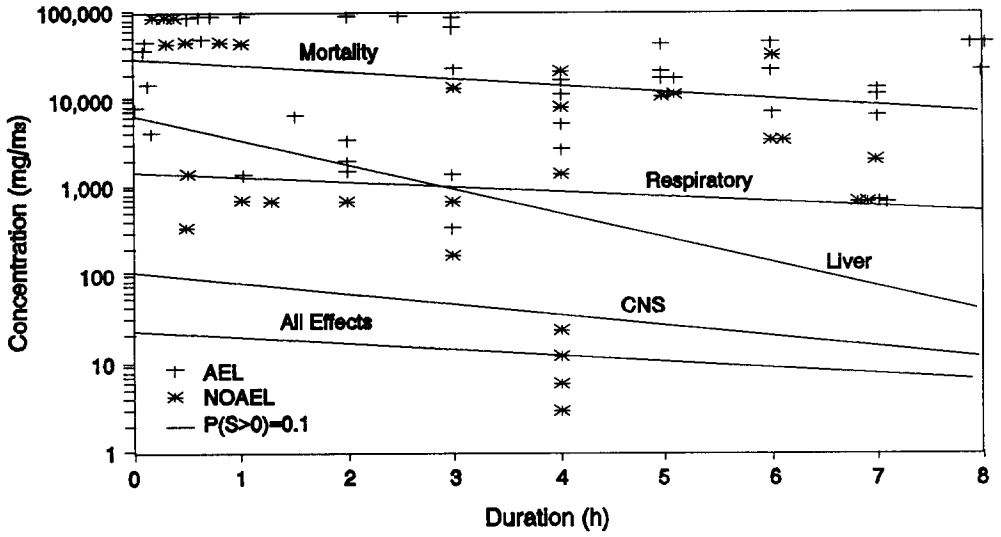


**Figure A-13. Categorical regression analysis of tetrachloroethylene: acute effects. Individual regression lines are based on model fit for all observations of specified effects. Each point is an independent exposure group defined as a specific concentration, duration, species, strain, and sex in a study.**

Source: Guth et al. (1991).

mechanistic processes are involved for each of those types. As with the other alternative approaches discussed, application of interspecies dosimetry adjustments and UFs for data extrapolation are also warranted.

Development of this model application should also address the appropriateness of combining data of different durations. For the RfC, subchronic and chronic data are of interest to estimate "lifetime" effects. Consideration of temporal aspects of toxicity (see Section 4.3.2) is required. The linearity of responses with exposures is often assumed, but rarely investigated over "lifetime" bioassays.

# APPENDIX B

# CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL EPIDEMIOLOGICAL STUDIES[1]

Human data obviate the need for interspecies extrapolation and thus represent valuable information to dose-response assessment. Scientific controversy sometimes surrounds the interpretation and significance of results when the nature of the study was not experimental. Guidelines for good epidemiology practices, documentation guidance, and guidance on preparation of quality assurance studies for epidemiologic studies have been developed that provide a surrogate to good laboratory practice standards aimed at laboratory animal studies. These guidelines address the process of conducting epidemiologic studies in order to ensure the quality and integrity of the data and to provide adequate documentation of the research methods.

The criteria for assessing the quality of individual epidemiologic studies provided herein are adapted from these guidelines and a number of sources. These criteria are intended to serve as guidance on the evaluation of the quality of the practice with which the study was conducted. These criteria fundamentally represent good scientific practice and thereby impart an index as to the level of uncertainty when utilizing a particular study for dose-response assessment. It is recognized that in some cases, information is not available to ascertain whether all the criteria have been met, in which case judgment is necessary. For example, the typical peer-reviewed journal article lacks some of the information provided in a detailed study report.

1. The relationships, roles, and responsibilities of the organizations and/or individuals sponsoring or conducting the study should be defined in writing. Sponsorship and funding sources should be acknowledged.

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[1]Adapted from: Interagency Regulatory Liason Group (1981), Lebowitz (1983), American Thoracic Society (1985), Pickrel et al. (1986), and Chemical Manufacturers Association's Epidemiology Task Group (1991).

2. A critical review of the relevant literature to evaluate applicable findings should be provided. The review should encompass laboratory animal and human experiments, clinical studies, vital statistics, and previous epidemiologic investigations. The review should be sufficient to identify potential confounders and effect modifiers.

3. The objectives, specific aims, and rationale of the study should be clearly stated.

4 The overall research design, strategy, and rationale for choosing the proposed study design should be described in relation to the objectives. Limitations of the study design should also be stated. Underlying assumptions and limitations of the design also should be given.

5. Clear definitions of health outcomes, exposure, other measured risk factors, and selection criteria should be provided, as appropriate, for the study population and comparison group (nonexposed and/or referent), morbidity and mortality cases. The study population and comparison group description should include the specific population from which they were drawn and the method of selection. The rationale and criteria for inclusion or exclusion of participants in the study should be given, particularly for exposure classifications. The appropriateness and limitations of the comparison group should be discussed. The extent to which the choice of subjects depended on existing or specially developed record systems, and implications of this upon the analysis, should be considered. The steps taken to ensure confidentiality of the subjects should be accounted for.

6. Data sources for exposure, health status, and risk factors should be described (e.g., questionnaires, biological measurements, exposure/work history record reviews, or exposure/disease registries). The limitations of these sources should be described.

7. Methods of data collection should be described in detail, because these procedures will influence the derived interpretation and inferences. This should include a description of, or reference to, methods used to control, measure, or reduce various forms of error

(e.g., bias due to misclassification, interviewer, or confounding factors) and their impact on the study. The validity (accuracy) and reliability (reproducibility) of the methods used to determine exposure should be stated. Response rates, including reasons for implications of differing rates, should be given. The direction and possible magnitude of any bias introduced into the study as a result of these rates should be described. The procedures used for following the study, methods to ensure completeness, and length of follow-up for each group or subgroup must be included. Other validity checks (e.g., avoiding bias by the independent ascertainment and classification of study variables, such as blind reading of histologic slides or clerical processing of data) also should be included.

8. Major demographic and anthropometric confounding factors should have been accounted for, such as age, sex, ethnic group, socioeconomic status, smoking status, and occupational exposure. The methods employed for these adjustments and their limitations should be discussed. Temperature, season, and day of the week are particularly important for acute studies of respiratory effects and also should be accounted for.

9. The procedures and statistical methods used to describe and analyze the data, estimate parameters, or test specific hypotheses should be presented. References and/or specific formulae also should be given for the statistical tests and for any programming procedures or packages that were applied. The underlying assumptions and potential bias of the statistical methods should be stated. Explicit description of any method used to account for confounding factors (e.g., adjustment or matching) should be described explicitly. This includes methods to account for missing data, such as from nonresponse, attrition, or loss-to-follow-up. When reporting hypothesis tests, the measure of effect, statistical significance, power, and other criteria (e.g., one- versus two-tailed test rationale) should be given. Procedures for obtaining point estimates and their standard errors and/or confidence intervals should be given when using estimation.

10. Criteria for interpreting results should be discussed, including the influence of the limitations of the design, data sources, and analytic methods. Criteria for assessing biologic plausibility, internal and external consistency of the findings, and causal inference (see Appendix C) should be stated.

Often the detailed laboratory reports and documentation of studies are evaluated along with peer-reviewed papers when evaluating data for derivation of an RfC. Quality assurance and guidelines have been developed to ensure that essentially the same requirements provided herein are met and these can be used to assess the quality and data integrity of completed studies (Pickrel et al., 1986; Chemical Manufacturers Association, 1991). Each study should have a written protocol that was approved before the study began. Data are usually considered draft unless the final report has been signed. The following are suggested items for inclusion in a written protocol that should accompany any formal report (Chemical Manufacturers Association's Epidemiology Task Group, 1991).

A. Descriptive title.
B. The names, titles, degrees, addresses, and affiliations of the study director, principal investigator, and all co-investigators.
C. The name(s) and address(es) of the sponsor(s).
D. An abstract of the protocol.
E. The proposed study tasks and milestones, including study approval date (date protocol signed by all signatories), study start date (first date the protocol is implemented), periodic progress review dates, and completion date.
F. A statement of research objectives, specific aims, and rationale (See criteria number 3 above).
G. A critical review of the relevant literature to evaluate applicable findings (See criteria number 2 above).
H. A description of the research methods, including:
    1. The overall research design, strategy, and rationale for choosing the proposed study design.
    2. The data sources for exposure, health status, and risk factors.

3. Clear definitions of health outcomes, exposure, and other measured risk factors as well as selection criteria, as appropriate, for exposed and nonexposed persons, morbidity or mortality cases, and referent groups.
4. Projected study size and, if appropriate, statistical power.
5. The methods to be used in assembling the study data.
6. Procedures for handling the data in the analysis.
7. Methods for data analysis.
8. Major limitations of the study design, data sources, and analytic methods.
9. Criteria for interpreting the results.

I. A description of plans for protecting human subjects.

J. A description of, or reference to, quality assurance and quality control procedures for all phases of the study. As appropriate, include certification and/or qualifications of any supporting laboratory or research groups.

K. A description of plans for disseminating and communicating study results.

L. Resources required to conduct the study.

M. The bibliographic references.

N. Addenda, as appropriate, including correspondence, collaborative agreements, institutional approval, and samples of the informed consent forms, questionnaires, and representative samples of other documents to be used in the study.

O. A dated protocol review and approval sign-off sheet for the study director, principal investigator, co-investigators, and all reviewers.

P. Dated amendments to the protocol.

# APPENDIX C

# CRITERIA FOR CAUSAL SIGNIFICANCE

Statistical methods cannot establish proof of a causal relationship but can define an association with a certain probability. The causal significance of an association is a matter of judgment that goes beyond any statement of statistical probability. To assess the causal significance of an air toxicant and a health effect, a number of criteria must be used, no one of which is pathognomonic by itself. These criteria include the following:

- Consistency (reproducibility) of the association. Causal inferences are strengthened when a variety of investigators have reproduced the findings under a variety of circumstances.
- Strength of the association. The larger the calculated relative risk, the greater the likelihood that the observed association is causal.
- Specificity of the association. Causality is more likely if a particular exposure is associated with only one illness and vice versa. This guideline rarely applies to air pollution research, in which all the diseases of major concern are multifactorial.
- Temporal relationship of the association.
- Coherence of the association. An epidemiologic inference of causality is greatly strengthed when it conforms to knowledge concerning the biologic behavior of a toxicant and its mechanism of action. This evidence may be obtained from clinical research or toxicologic studies.
- Dose-response relationship.

# APPENDIX D

# ADVERSE HUMAN RESPIRATORY HEALTH EFFECTS

These criteria were developed to assist in the interpretations of the epidemiologic literature on what constitutes an adverse respiratory health effect of air pollution. Adverse human health effects caused by air pollution are listed in hierarchical order, with the most severe at the top and the least severe at the bottom. The reader is referred to the American Thoracic Society (1982, 1985, 1986, 1993) guidelines, Epler et al. (1980), and Chan-Yeung (1987) for more detailed discussion as to what constitutes respiratory impairment in humans and to Appendix E for a discussion of pulmonary function testing data.

1. Increased mortality. ("Increased", as used here and subsequently, means significantly [$p < 0.05$] increased above that recorded in some standard, comparable population. In selected situations, $p < 0.1$ may be appropriate.)
2. Increased incidence of cancer.
3. Increased frequency of symptomatic asthmatic attacks.
4. Increased incidence of lower respiratory tract infections.
5. Increased exacerbations of disease in humans with chronic cardiopulmonary or other disease that could be reflected in a variety of ways, including the following:

    - Less able to cope with daily activities (i.e., shortness of breath or increased anginal episodes);
    - Increased hospitalizations, both frequency and duration;
    - Increased emergency ward or physician visits;
    - Increased pulmonary medication; and
    - Decreased pulmonary function.

6. Reduction in forced expiratory volume at one second ($FEV_1$) or forced vital capacity (FVC) or other tests of pulmonary function such as the following:

   - Chronic reduction in $FEV_1$ or FVC associated with clinical symptoms.
   - A significant increase in number of persons with $FEV_1$ below normal limits; chronically reduced $FEV_1$ is a predictor of increased risk of mortality. Transient or reversible reductions that are not associated with an asthmatic attack appear to be less important. It should be emphasized that a small but statistically significant reduction in a population mean $FEV_1$ or $FEV_{0.75}$ is probably medically significant to them, but when diluted with the rest of the population, the change appears to be small.
   - An increased rate of decline in pulmonary function ($FEV_1$), relative to predicted value in adults with increasing age or failure of children to maintain their predicted $FEV_1$ growth-curve. Such data must be standardized for sex, race, height, and other demographic and anthropometric factors.

7. Increased prevalence of wheezing in the chest, apart from colds, or of wheezing most days or nights. (The significance of wheezing with colds needs more study and evaluation.)
8. Increased prevalence or incidence of chest tightness.
9. Increased prevalence or incidence of cough/phlegm production requiring medical attention.
10. Increased incidence of acute upper respiratory tract infections that interfere with normal activity.
11. Acute upper respiratory tract infections that do not interfere with normal activity.
12. Eye, nose, and throat irritation that may interfere with normal activity (e.g., driving a car) if severe.
13. Detection of odors.

# APPENDIX E

# GUIDANCE ON PULMONARY FUNCTION TESTING

The two primary functions of the lung, oxygenation of mixed venous blood and removal of carbon dioxide from that same blood, depend on the integrity of the airways, the vascular system, and the alveolar septa. Inhaled toxic chemicals can affect the integrity of all three of these components. Ideally, tests would be designed to assess the integrity and functional relationships of these structures separately. However, because this is often difficult, many pulmonary function tests evaluate the status of these structural components in an indirect, and often overlapping, way. The myriad of tests include those of pulmonary ventilation, mechanics, distribution, diffusion, and ventilation/blood flows.

During the last three decades, lung function tests have evolved from tools for physiologic study to clinical tools widely used in assessing respiratory status. It has become common to evaluate the results of lung function tests in terms of whether or not they are considered to be within a "normal" range (i.e., represent an "adverse" effect or not). These interpretations are increasingly becoming the basis of dose-response assessments. All clinical measurements, including pulmonary function tests (PFT) are subject to (1) technical variation related to instrument, procedure, observer, subject, and their interactions; (2) biologic variation; and (3) variation caused by dysfunction or disease, the focus of interest for dose-response assessment. Therefore, interpretation of PFT requires establishing the variation of interest (the signal) and its relation to the other sources of variation (the noise).

To maximize the clinical value of lung function testing, the American Thoracic Society (ATS) has outlined the steps necessary to achieve standardization: (1) equipment performance, validation, and quality control; (2) subject performance; (3) measurement procedures to determine acceptability and reproducibility; and (4) reference values and interpretation. These steps form the basis of the criteria outlined here and can loosely be applied to the evaluation of tests on both human and laboratory animals, although in most instances, subject performance is not voluntary in the laboratory animals. Adherence to the

available guidance and recommendations discussed herein should help to ensure that changes in lung function over time and from certain exposures can be correctly interpreted and analyzed without reservation regarding their accuracy and quality.

For more detailed discussion on selection of reference values, interpretative strategies and standardization approaches for pulmonary function testing in humans, the reader is referred to American Thoracic Society (1979; 1987a,b; 1991), Gardner et al. (1986a,b,c), McKay (1986), McKay and Lockey (1991), Folinsbee (1988), Clausen (1982) and Ruppel (1979). This appendix discusses considerations affecting the evaluation of PFT performed on human subjects. For a more detailed discussion as to what constitutes respiratory impairment in humans, the reader is referred to Appendix D and to the American Thoracic Society (1982, 1986, 1993) guidelines, Epler et al. (1980), and Chan-Yeung (1987). Although some of the general concepts are applicable to laboratory animals, some of the procedures and definitions of PFT for laboratory animals are different and these are highlighted at the end of each section. For more detailed discussion of the interpretations and limitations of pulmonary function testing in laboratory animals and their correlates to human PFT, the reader is referred to Costa et al. (1992); Costa and Tepper (1988); Mauderly (1989), and Costa (1985).

## E.1 GENERAL DEFINITIONS

This section provides the definitions of (1) the tests commonly used to evaluate pulmonary function and (2) the basic ventilatory defects.

### E.1.1 Common Pulmonary Function Tests in Humans

Figure E-1 is a diagrammatic representation of the various lung volumes and capacities based on a typical spirogram and Table E-1 provides the description, determination technique, and significance of each in the context of possible diagnostic use. There are some causes for changes in these tests (e.g., limitation of the movement of the diaphragm by pregnancy, thoracic surgery, or neuromuscular disease) that are not addressed by these comments. It should be recognized that this table is very general and any decision on the significance of

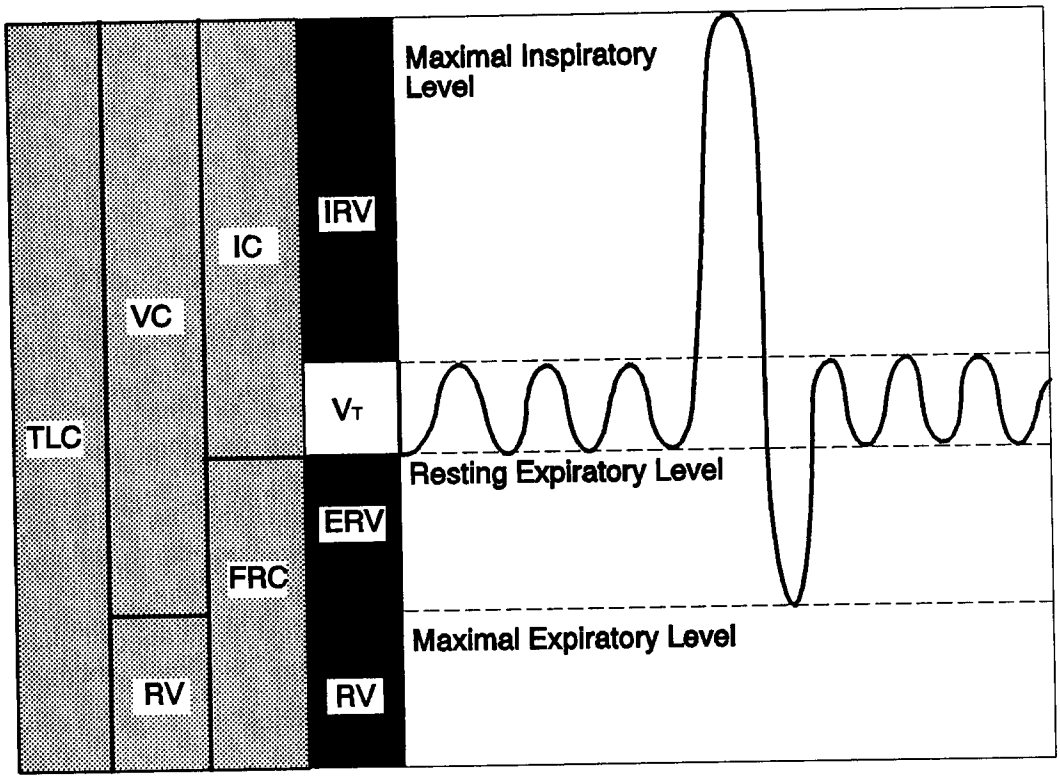


**Figure E-1. Lung volumes and capacities. Diagrammatic representation of various lung compartments, based on a typical spirogram. TLC, total lung capacity; VC, vital capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; $V_T$, tidal volume; IRV, inspiratory reserve volume; ERV, expiratory reserve volume. Shaded areas indicate relationships between the subdivisions and relative sizes as compared to the TLC. The resting expriatory level should be noted, since it remains more stable than other identifiable points during repeated spirograms, hence is used as a starting point for FRC determinations, etc.**

Source: Ruppel (1979).

abnormality observed in any given study depends heavily on the circumstances under which the testing was performed.

Pulmonary mechanics tests include the forced vital capacity (FVC), the forced expiratory volume ($FEV_T$) and the forced expiratory flow at 25 to 75% exhaled FVC ($FEF_{25-75\%}$). All values should be expressed using volumes corrected to body conditions (BTPS): normal body temperature (37 °C), ambient pressure (mm Hg) saturated with water vapor.

## TABLE E-1. DEFINITION OF VARIOUS PULMONARY FUNCTION TEST VOLUMES AND CAPACITIES

| Volume or Capacity | Description | Technique | Significance |
|---|---|---|---|
| TLC | Total Lung Capacity. The amount of air contained in the lungs at the end of a maximal inspiration. | Usually calculated by combination of other specific lung volumes (e.g., FRC + IC, VC + RV). | TLC is decreased in edema, atelectasis, pulmonary congestion, and restrictive diseases. The TLC may be normal or increased in bronchiolar obstruction with hyperinflation and in emphysema. |
| VC | Vital Capacity. The largest volume measured on complete expiration after the deepest inspiration without forced or rapid effort. | Vital capacity is measured from maximal inspiration to maximal expiration ("I-E") or maximal expiration to maximal inspiration ("E-I")[1]. | A decrease in VC may be caused by a loss of distensible lung tissue (e.g., bronchiolar obstruction or pulmonary congestion). |
| RV | Residual volume. The volume of air remaining in the lungs at the end of a maximal expiration. | RV must be measured indirectly as a subdivision of the FRC, using $N_2$-washout (open-circuit) method or tracer gas dilution (closed circuit). | Increases in RV are characteristic of emphysema and chronic air trapping, as well as chronic bronchial obstruction. RV is typically decreased in restrictive diseases, particularly those associated with extensive fibrosis, such as sarcoidosis, asbestosis, and silicosis. RV may also be decreased in diseases that occlude many alveoli (e.g., pneumonia). |
| IC | Inspiratory capacity. The largest volume of air that can be inspired from the resting expiratory level. | IC is measured by inhaling maximally from the resting expiratory level or estimated by: VC − ERV. | Changes in the absolute volume of IC usually parallel increases or decreases in the VC. Compensatory hyperventilation normally "dips into" the inspiratory capacity because both the end-inspiratory and end-expiratory levels are altered. |
| FRC | Functional residual capacity. The volume of air remaining in the lungs at the end-expiratory level. | See RV. | An increased FRC represents hyperinflation that may result from emphysematous changes, asthmatic or fibrotic bronchiolar obstruction. FRC is typically decreased in restrictive diseases, particularly those associated with extensive fibrosis, such as sarcoidosis, asbestosis, and silicosis. FRC may also be decreased in diseases that occlude many alveoli (e.g., pneumonia). |

**TABLE E-1 (cont'd). DEFINITION OF VARIOUS PULMONARY FUNCTION TEST VOLUMES AND CAPACITIES**

| Volume or Capacity | Description | Technique | Significance |
|---|---|---|---|
| IRV | Inspiratory reserve volume. The largest volume of air that can be inhaled from the tidal inspiratory volume. | IRV is measured by inhaling maximally from the tidal inspiratory volume. | Changes parallel to those in VC. |
| $V_T$ | Tidal volume. The volume of air inspired or expired during each respiratory cycle. | $V_T$ is measured directly by simple spirometry. The volume change is measured from the excursions of normal breathing. Because no two breaths are identical, the $V_T$ inhaled or exhaled should be measured for at least 1 minute and then divided by the rate to determine the average. | $V_T$ is not an adequate indicator of alveolar ventilation and should not be considered outside the context of rate and minute volume. |
| ERV | Expiratory reserve volume. The largest volume of air that can be expired from the end-expiratory level. | ERV is measured by exhaling maximally from the resting expiratory level or estimated by: VC − IC. | Changes parallel to those in VC. |

[1]The closed circuit technique enables evaluation of whether a maximum inspiration was achieved prior to expiration for the "I-E" maneuver (McKay and Lockey, 1991).

The FVC is the volume (liters) of air that can be exhaled as forcefully and rapidly as possible after a maximal inspiration. The test's validity depends heavily on patient effort and cooperation (see footnote to Table E-1). The $FEV_T$ is the volume of air exhaled over a specified time interval (liters per seconds) during the performance of a FVC. The time interval (in seconds) is stated as a subscript to FEV. An interval in common use is the $FEV_1$, the volume expired at 1 s. The $FEF_{25-75\%}$ is the mean forced expiratory flow during the middle half of the FVC, formerly called the maximal midexpiratory flow (MMEF). Figure E-2 shows a typical volume-time curve (spirogram) for the FVC maneuver and various $FEV_T$ are indicated.

Bronchial responsiveness is an integrated physiologic mechanism involving airway epithelium, nerves, mediators, and bronchial smooth muscle. Bronchoprovocation challenge testing (BPCT) involves evaluating the changes in FVC, $FEV_T$, and $FEV_1$/FVC ratio after exposure to either specific or nonspecific agents capable of producing bronchoconstriction.

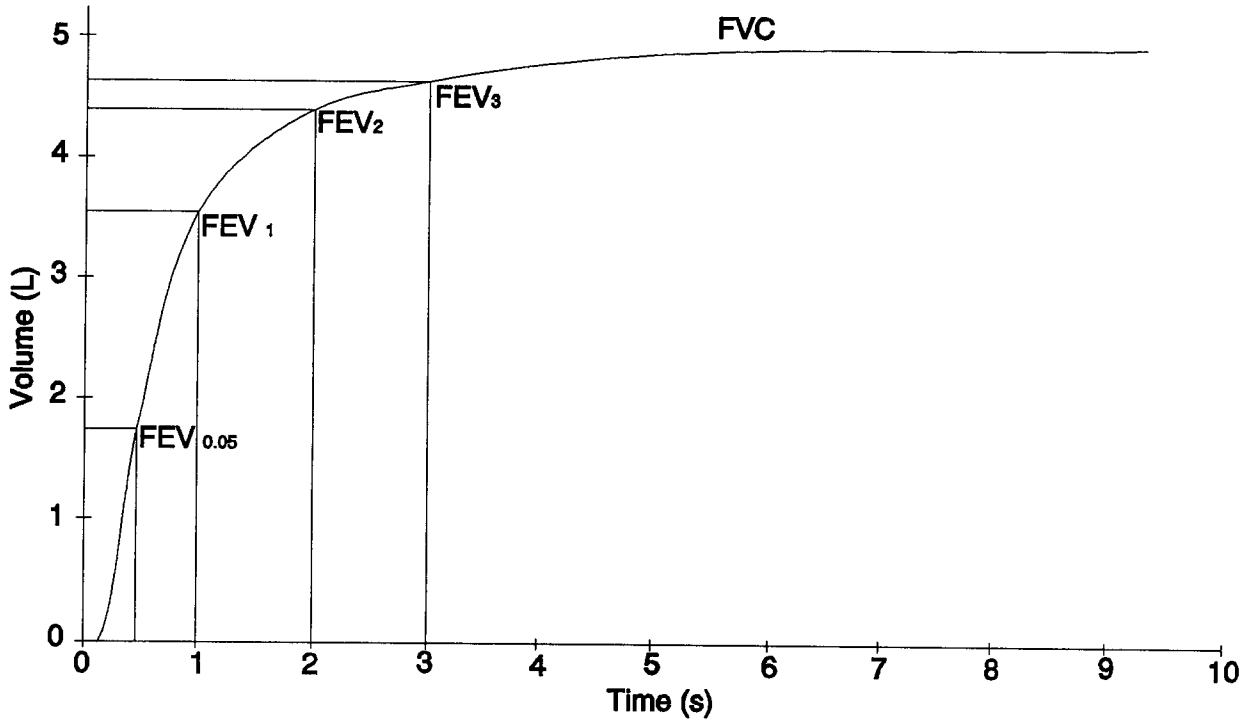


**Figure E-2. Volume-time plot (spirogram) of the forced vital capacity (FVC) maneuver. The subject exhaled as forcefully and rapidly as possible from maximal inspiratory level. Forced expiratory volume (FEV) as various time intervals are indicated.**

Parasympathomimetic drugs, such as methacholine and carbachol, are used as nonspecific agonists because they cause bronchoconstriction by stimulating acetylcholine receptors located directly on airway smooth muscle. Histamine is another commonly used bronchoconstricting agent. Although its mechanism of action is somewhat controversial, it probably acts indirectly by stimulating cholinergic nerve endings as well as having a direct effect via histamine receptors on airway smooth muscle. Specific agents include common antigens or chemicals such as the isocyanates that may provoke immediate, delayed or dual pulmonary responses that may not resolve spontaneously. Guidelines for standardization have been developed for bronchial inhalation challenges with the nonspecific agonists such as methacholine (Cropp et al., 1980) and adherence to these guidelines should be considered when evaluating such data. Bronchoprovocation challenge testing (BCPT) with specific agents requires more time, expense, and sophisticated equipment and remains more in the realm of research than does nonspecific BCPT, but can also be an extremely useful diagnostic

aid when performed by a quality laboratory. Factors influencing agent-specific BPCT have been discussed elsewhere (McKay, 1986).

Response to bronchodilating agents may also be measured. The within-individual difference in response to different bronchodilators is variable. Because the correlation between bronchoconstriction and bronchodilator response is imperfect, it is not possible to infer with certainty the presence of one from the other. There is no clear consensus on what constitutes reversibility in subjects with airflow obstruction (American Thoracic Society, 1991), however, 20% reversibility is generally believed to be consistent with asthma.

Carbon monoxide diffusing capacity ($DL_{CO}$) measures all the factors that affect the diffusion of a gas across the alveolo-capillary membrane. Traditional units are mL CO/min/mm Hg at STPD (standard conditions: 0 °C, barometric pressure of 760 mm Hg, 0 mm Hg water pressure). Steady-state or rebreathing techniques are commonly used for human testing. But the single-breath technique ($DL_{COsb}$) is also commonly used. In general, $DL_{CO}$ is decreased in alveolar fibrosis (e.g., as associated with asbestosis or berylliosis) or interstitial edema. Carbon monoxide diffusing capacity is also decreased in emphysema because of the decrease in alveolar surface area, loss of capillary bed, increased distance from the terminal bronchiole to the alveolocapillary membrane, and the mismatching of ventilation and blood flow. Guidance on standardization has been published elsewhere (American Thoracic Society, 1987b).

The nitrogen washout test measures the concentration of nitrogen in alveolar gas at the end of breathing 100% oxygen for a prescribed period of time (e.g., 7 min). The value is recorded as a percentage of nitrogen. The test is used to determine lung volumes (e.g., the FRC and RV). The FRC and RV are often increased in diseases in which there is an increased airway resistance such as emphysema, chronic bronchitis, and asthma. The RV is raised in these conditions chiefly because airway closure occurs at an abnormally high lung volume. A reduced FRC and RV are often seen in conditions of reduced lung compliance, for example, in diffuse interstitial fibrosis. In this case, the lung is "stiff" and tends to recoil to a smaller RV.

### E.1.1.1 Common Pulmonary Function Tests in Laboratory Animals

The conceptual framework for analysis of pulmonary function is quite similar for laboratory animals with the following noteworthy differences:

1. The relevance of the various ATS guidelines is questionable since in many instances they specifically define terms, procedures, and equipment only as they relate to humans. The reviews of Costa (1985), Costa and Tepper (1988), Mauderly (1989), and Costa et al. (1992) put most of the information presented here for the human in the context of small laboratory animals with the various caveats and limitations. The reader is referred to these reviews for more detail than can be provided in these guidelines. For example, if the animal test is done under anesthesia, its body temperature will fall. Unless this measure is monitored and used in the computation of the BTPS adjusted measure (assumed to be 37 °C), the data can differ between studies. Many investigators use actual body temperature (approximately 35 °C) as the BTPS basis so that temperature need not be monitored.

2. The tests described for humans generally require the use of nomogram or other standardized tables based on sex, age, height, and weight of the test subject to compare and determine "normalcy". Laboratory animal studies almost always require the use of comparable control groups (based on the same specio-promorphic considerations) against which determination of effect is established. Anomaly or effect is based on statistical grounds for the group and rarely for the individual (except as part of the overall interpretation).

3. Measures of maximal lung volume in laboratory animals are determined by imposed pressures derived from allometric evaluations of cross-species data, not effort. Since these animal measures are determined under anesthesia, volition is eliminated and the static mechanics of the system can be established. The forced vital capacity measure in the rodent is created differently from that of the human. The interpretation is similar, but reductions in these species respective volumes can differ because of pain in the human maneuver (e.g., after ozone exposure) which the animal will not feel. Nuances can be important. Similarly, the measure of FRC in laboratory animals and humans is different based ont he mechanisms establishing this volume. In the human, the volume is based on apposed recoil of the lung and chest wall. In animals with compliant chests this is not the case. Rather, it is set by breathing mechanics and central expiratory control (turned off during anesthesia). Hence, true comparison is difficult. In fact, because the rodent lung has the ability to in part regenerate after acute injury, the FRC response may be the reverse of that of the human (larger than normal instead of smaller). The $DL_{CO}$ can respond in much the same manner.

4. Airway reactivity in the rodent is measured in many different ways. Almost none of the these directly parallels the human but the overall interpretations are the same. However, there can be toxicant differential effects in animals when the agonist is delivered to the lung directly versus intravenously. It should be noted

that even within the human study community that the methods used for the testing of airway reactivity differ significantly from laboratory to laboratory. Standardization is more commonplace in true clinical test laboratories than in empirical-clinical study laboratories.

## E.1.2 Interpretation of Pulmonary Function Tests and Basic Ventilatory Defects

The vital capacity (VC), $FEV_1$, and $FEV_1$/VC ratio are the basic parameters used to interpret spirometry. Although FVC is often used in place of VC, it is preferable to use the largest VC, whether obtained on inspiration (IVC), slow expiration (EVC), or forced expiration (FVC) for clinical testing. Limiting primary interpretation of spirometry to three variables avoids the problem of simultaneously examining a multitude of measurements to see if any abnormalities are present, a procedure that will lead to an inordinate number of "abnormal" tests (American Thoracic Society, 1991). As discussed in other sections of this appendix, the first step in interpreting lung function data should be the evaluation of the quality of the testing. Further, tests interpreted without additional clinical information are limited in their utility to be definitive. Consideration must also be given to (1) the level of reporting and control of technical variation (Section E.2) and (2) the selection of reference values and statistical techniques used to generate predictive values that may be used for interpretation (Section E.4).

### E.1.2.1 Definition of an Obstructive Defect

An obstructive ventilatory defect may be defined as a disproportionate reduction of maximal airflow from the lung with respect to the maximal volume (VC) that can be displaced from the lung. It indicates airflow limitation and implies airway narrowing during expiration. The earliest change associated with flow limitation in small airways is thought to be slowing in the terminal portion of the spirogram even when the initial phase is unaffected. This slowing is reflected in a proportionally greater reduction in the instantaneous flow measured after 75% of the FVC has been exhaled ($FEF_{75\%}$) or in the $FEF_{25-75\%}$, than in the $FEV_1$. Abnormalities in these midrange flow measurements during a forced exhalation are, however, not specific for small airway disease and, though suggestive, should not be used to diagnose small airway disease in individual patients. As airway disease becomes more

advanced and/or more proximal airways become involved, earlier time segments of the forced expiratory maneuver such as the $FEV_1$ will become reduced out of proportion to the reduction in the VC (American Thoracic Society, 1991).

The $FEV_1/VC$ is recommended as the primary test for distinguishing obstructive from nonobstructive patterns. The $FEF_{25-75\%}$ may be used to confirm the presence of airway obstruction in the presence of a borderline $FEV_1/VC$. The severity of airway obstruction should be based on the $FEV_1$ rather than the $FEV_1/VC$ (American Thoracic Society, 1991).

### E.1.2.2 Definition of a Restrictive Defect

A restrictive ventilatory defect is characterized physiologically by a reduction in TLC. The presence of a restrictive ventilatory defect is inferred when VC is reduced and the $FEV_1/FVC$ is normal or increased. However, severe airflow limitation is another common cause of a reduced VC, either because airflow is so slow the subject can not continue to exhale long enough to complete emptying or because airways collapse. Also, a small VC with a normal $FEV_1/VC$ will occasionally be observed in patients with a normal TLC. Thus, if there is a contradiction between VC and TLC in defining restriction, the classification should be based on the TLC (American Thoracic Society, 1991).

### E.1.2.3 Interpretation of Laboratory Animal Tests

A notable difference for interpretation of laboratory animal pulmonary function testing is that these studies typically require cohort control groups because of the many influences on the animal that can not be standardized in textbook nomograms. The reader is referred to the reviews of Costa (1985), Costa and Tepper (1988), Mauderly (1989), and Costa et al. (1992) for important distinctions from human clinical interpretations.

## E.2 TECHNICAL SOURCES OF VARIATION (INSTRUMENTATION)

Maximizing the usefulness of spirometry for clinical, diagnostic, or epidemiologic purposes depends on a number of factors that begins with equipment selection. Because spirometry involves effort-dependent maneuvers that require careful patient/subject instruction, understanding, coordination, and cooperation, performance recommendations are

also an important component of ensuring accurate testing. This section discusses specific guidance available on testing equipment, testing performance, quality control, and technician training. This guidance is intended to serve as a framework by which to evaluate the level of certainty in the use of reported spirometry data.

## E.2.1 Equipment

Measurement of the deterioration of pulmonary function as an effect of exposure to a toxic chemical may be erroneous if inaccurate spirometers (or other instrumentation) or less sensitive if imprecise spirometers are used. Thus, equipment selection and maintenance is pivotal to ensuring accurate test results. The accuracy of a spirometer systems depends on the resolution (i.e., the minimal detectable volume or flow) and linearity of the entire system—from volume or flow transducer to recorder, display, or processor. Studies should state that the equipment was validated as meeting ATS recommendations. Mention should also be made that equipment quality control procedures were routinely performed, including preventive maintenance, calibration checks, verification and that a quality assurance program was in place to ensure accurate spirometry and test results (American Thoracic Society, 1991; Gardner et al., 1986a,b). Attention must be given to the spirometer temperature where the tests are performed and values reported in BTPS. Quality control should at least include strict adherence to ATS guidelines for equipment performance and calibration (American Thoracic Society, 1991) and additional equipment recommendations have been made by McKay and Lockey (1991).

Measurement procedures have been recommended to ensure that uniform methods are used and that comparable results are obtained (American Thoracic Society, 1991). Medical surveillance and epidemiological studies may require more stringent guidelines to ensure the higher level of quality needed to detect changes from one year to another (McKay and Lockey, 1991). Measurement procedures include how to perform specific maneuvers and thus also define equipment requirements as well. For example, if a test procedure should be carried out for at least a specified amount of time, the spirometer should at a minimum be able to compile data for that duration. Other spirometry system recommendations related to performance procedures include specifications on volume range and accuracy, flow range, resistance and back pressure, time scale (paper speed), volume scale, flow:volume scale,

display axes orientation, and the type of signal used to test the performance for a given maneuver.

### E.2.2 Procedure Performance and Measurement

Performance recommendations are an important component of testing because PFT involves effort-dependent maneuvers that require careful patient/subject instruction, understanding, coordination, and cooperation. The largest single source of within-subject variability is improper performance of the maneuvers (American Thoracic Society, 1991). The performance recommendations involve obtaining a sufficient number of maneuvers that are of adequate quality and then determination as to whether these acceptable maneuvers are reproducible. Once maneuvers have been performed, measurement procedures are included to help ensure that uniform methods are used and that comparable results are obtained. Interpretations of spirometry should include a statement about test quality before any other interpretation is rendered.

Guidance on how to perform specific maneuvers (i.e., the VC, FVC, $FEV_T$, and $FEF_{25-75\%}$) include recommendations on satisfactory start of test criteria, end of test criteria, subject instruction, minimum maneuver time, maximum number of maneuvers, acceptability criteria, use of noseclips, sitting versus standing position, reproducibility criteria, test result selection, and result reporting. If a study does not explicitly state in the methods section that ATS-recommended procedures were performed, the description of the methods for the maneuvers should be compared against the available recommendations (American Thoracic Society, 1991; McKay and Lockey, 1991) to ascertain their credibility.

Proper training of persons administering PFT is the single most important component of a respiratory surveillance program (McKay and Lockey, 1991). Spirometry is not a set of simple procedures to be performed by untrained or minimally trained individuals. The persons administering PFT must do so with skill and understanding. The technician must be able to (1) adequately prepare the subject for testing; (2) identify any preexisting contraindications or reasons to postpone testing; (3) properly instruct, demonstrate, and coach the subject regarding proper technique; and (4) visually inspect each maneuver tracing for validity. The technician must be able to correct and adjust technical problems that may occur and be capable of responding to questions that may arise. The technician must also be

capable of accurately performing hand measurements and calculation and be able to confirm the adequacy of software used for automated calculations. This person should also be able to interpret tests and recognize the effect submaximal effort has on the interpretation process. Studies should state that qualified personnel were used and that a program was in place to evaluate the personnel periodically in order to ensure that accurate and reliable test results were obtained. Testing of commercially available spirometers showed that a major source of errors was in computer software. Due to the increased use of automated systems and computers in pulmonary laboratories, the ATS published "Computer Guidelines for Pulmonary Laboratories" (Gardner et al., 1986c).

### E.2.3 Technical Sources of Variation in Laboratory Animal Testing

Throughout this section, application to animal testing requires special considerations. The most notable are:

1. rapid responding plethysmographic and transducing equipment is required since most of the measures are in the 1-15 mL volume range and the flows perhaps as high as 150 mL/s with a response time of 40 ms or better, and

2. laboratory animals are typically anesthetized and orally or surgically (via the larynx) tracheotomized so that the nose and mouth have no influence.

## E.3 BIOLOGICAL SOURCES OF VARIATION

This section outlines sources of variation in PFT related to individual performance on the tests or to host factors, including environmental factors, of the individual tested. The factors are provided here for readers to be aware of as factors that should be controlled for (when possible) in studies that use PFT to index respiratory dysfunction. Some of these factors are explicitly incorporated in algorithms available to calculate normal values for various maneuvers and others are not (see Section E.4). Recommendations to control for some of these (e.g., recommended body position for most maneuvers) have been made to ensure consistency (American Thoracic Society, 1991).

The main sources of intraindividual variation of PFT are (1) body position, (2) head position, (3) effort dependency of maximal flows, and (4) circadian rhythms. The study design should include procedures that ensure consistency relative to these four factors.

The most important host factors that are responsible for interindividual variation in PFT are (1) sex, (2) size, and (3) age, which account for 30, 22, and 8%, respectively, of the variation between adults. Growth affects the relationship between indices of body size and spirometric measurements in children and adolescents. The relationship of ventilatory function to height from childhood through late adolescence to adulthood is not linear. Different prediction equation should be used for the sexes at all ages. Other sources of interindividual variation include (1) race and (2) past and present health.

Exposure to tobacco smoke is by far the most important factor known to alter lung function. A clear choice for the most appropriate method of adjusting spirometric indices for the effect of smoking is not readily evident from published data in which any of the following have been used: smoking status (current smoker or exsmoker), amount currently smoked, duration of smoking, and pack-years. Neglecting the correlation of some of these factors, (e.g., pack-years) with age can introduce errors in analyzing the effects of smoking. Smoking should be handled as an independent variable as its distribution in the reference population and its relation to other health indicators will affect any predictive regression terms calculated. Other environmental factors that contribute to interindividual variation include (1) geographic factors, (2) exposure to environmental and occupational pollution, and (3) socioeconomic status.

The reader is referred to the reviews of Costa (1985), Costa and Tepper (1988), Mauderly (1989), and Costa et al. (1992) for specific considerations in laboratory animals.

## E.4 REFERENCE VALUES: SOURCES, SELECTION AND STATISTICAL ISSUES[1]

Predicting the presence or absence of disease requires knowledge about the distribution of dysfunction in various disease states and the prior probability of disease. Subjects with similar characteristics for the major variables that affect lung function (sex, age, height, and race) can be grouped together in a stratum or a cell. Comparing the performance of an individual subject with the values generated from a reference population requires knowledge about the data in the appropriate cell (i.e., the number in the cell, measures of central

[1]Text adapted from American Thoracic Society (1991). Reader is referred to these guidelines for additional detail.

tendency such as the mean value, estimates of dispersion such as variance or standard deviation [SD], and information about the symmetry of the distribution). If the number of subjects in each cell is sufficient, PFT can be described by providing descriptors of the distribution such as mean and SD. Such tabulations are infrequently used for PFT because there are too many possible cells to consider all possible combinations of age and height.

Regression equations are an economical and efficient alternative method to describe expected values as a function of sex, height, and age. Regression techniques assume that PFT varies in a symmetric fashion about the mean value in each cell and that the variance about the mean is constant from one cell to another. The closer the distribution of PFT values come to symmetry or, better still, to a Gaussian distribution within cells, the more it is possible to take advantage of the equations. Distributions of $FEV_1$ and FVC in population studies are usually found to be close to Gaussian in the middle age range, not at the extremes. Ideally, publications describing reference populations should include, not only the prediction equations, but also a means of defining their lower limits. In the absence of explicit recommendations, a lower limit can be estimated from a regression model. For spirometry, values below the fifth percentile are taken as below the expected range (below the "lower limit of normal") and those above the fifth percentile are taken as within the expected range. This implies a 5% false positive misclassification, a rate generally considered acceptable.

The most commonly reported measures of how well regression equations fit the data are the square of the correlation coefficient ($r^2$) and the standard error of the estimate (SEE). The proportion of variation in the observed data explained by the independent variables is measured by $r^2$. The SEE is the average SD of the data around the regression line. Because these two statistics reflect average characteristics of the regression, $r^2$ and SEE may not reflect the ability of the equation to describe the tails of the distribution or the limits of "normal", and therefore are not sufficient criteria on which to choose the best equations to evaluate a population.

Linear regression is the most common but not the only model used to describe PFT data in adults. Such equations perform less well at the edges of the data distribution and in those cells where there are few data. Estimates are likely to be misleading if they go beyond the range of the independent variables used to create the equation.

Criteria for selecting reference values to be used fall into three categories: (1) methodologic, (2) epidemiologic, and (3) statistical. Reference values should be based on data obtained with the same instruments and methods comparable to those used for the population for which the reference values are being selected. The population from which the subjects are drawn should be similar with respect to age, height, sex, and ethnic composition to the population to whom the prediction values are to be applied. Prediction equations should use age, height, sex, and ethnic group as independent variables. For most uses, they should be based on cross-sectional studies of lifetime nonsmokers. Both biologic plausibility and simplicity in the model used to develop prediction equations are important issues in the selection of reference values. Other statistical aspects have been described above. Selected published reference equations for adult whites and blacks and scaling factors for blacks currently in use have been published. Studies should use the published reference equations that most closely describe the population being tested.

The practice in many clinical laboratories has been to classify values of FVC and $FEV_1$ less than 80% of predicted as abnormal. This fixed value has no statistical basis in adults.

Cross-sectional data are subject to a bias called "cohort" effect. A person who is 40 years of age today is different from one who became 40 two decades ago because of a variety of host and environmental factors. The age-related lung function deficit predicted from cross-sectional data tends to be greater than that predicted from longitudinal PFT data in adults and children. Prediction equations based on cross-sectional data are appropriate for determining the prevalence of PFT impairment in defined populations. They are less well-suited to determine age-related events including the incidence or progression of impairment. Reliance can be placed on the $FEV_1$ and VC for examining changes over time as they are the only spirometric variables that will consistently and correctly reflect the direction of the change in overall PFT. Difficulty remains, however, in determining whether a change is "real" or only a result of test variability. All PFT measurements tend to be more variable when made weeks to months apart than when repeated at the same session or even daily. It is more likely that a real change has occurred when there are a series of tests that show a consistent trend. As shown in Table E-2, significant changes, whether statistical or biologic, vary by parameter, time period, and the type of patient.

TABLE E-2. CHANGE IN SPIROMETRIC INDICES OVER TIME

| | Percent Changes Required To Be Significant | | |
|---|---|---|---|
| | FVC | $FEV_1$ | $FEF_{25-75\%}$ |
| Within a day | | | |
| Normal subjects | ≥5 | ≥5 | ≥13 |
| Patients with COPD | ≥11 | ≥13 | ≥23 |
| Week to week | | | |
| Normal subjects | ≥11 | ≥12 | ≥21 |
| Patients with COPD | ≥20 | ≥20 | ≥30 |
| Year to year | ≥15 | ≥15 | |

### E.4.1. Reference Values for Laboratory Animal Testing

The discussion above generally applies to laboratory animal studies with the exception noted above that the study design should include empirical control cohorts. Considerations for establishing such controlled studies are presented in the reviews of Costa (1985), Costa and Tepper (1988), Mauderly (1989), and Costa and Tepper (1992).

## E.5 INTERPRETIVE STRATEGIES: CONCEPTUAL ISSUES CONCERNING NORMALITY AND THE LIMITS OF NORMAL FOR DESIGNATING ADVERSE-EFFECT LEVELS

To draw inferences about the presence of disease from one test, the prior probability that the patient has the disease and the distributions of test values for subjects with and without the disease in question should ideally be known. Although this ideal is rarely met, understanding of the testing situation should be used to put an interpretation of PFT in proper perspective. The "normal" range only gives information about the distribution of test results in the healthy population from which they were derived. It says nothing about the true positive rate, the false negative rate, or the predictive power of a positive test.

As discussed in the preceding sections, consideration must be made of the appropriateness of the equipment, performance maneuvers, biologic variation and selection, including statistical procedures, used to derive normal reference values. In summary, studies

should indicate in the methods section the source of reference values used for their reports. Prediction equations for adults should include age, sex, and height as independent variables. It is preferable to choose reference values for both sexes from the same population source. Smoking status as an independent variable has been discussed in Section E.3. Altitude can be important in the selection of reference values for flow rates and for $DL_{CO}$. The equations should come from studies that present lower limits of normal or present information from which such lower limits can be calculated. In general, the prediction equations should not be extrapolated for ages or heights beyond those covered by the data on which they are based. The use of 80% of predicted for a lower limit of normal for adult PFT maneuvers is not recommended. Because of unexplained differences between published reference values, no one set of reference values is likely to be applicable to all studies performed. It is preferable that studies performed on populations in North America use reference values based on North American populations. European studies should use reference values based on European populations.

If there are any reasons to suspect the quality of the test performance, specific designation of adverse effect levels should be avoided. Dysfunction discovered under these conditions should indicate only the need for more definitive testing. General definitions of respiratory dysfunction are provided in Section E.1.2.2, but determination of the severity or degree of dysfunction must be made in the context of the other considerations discussed above, particularly the appropriateness of the reference values and statistical procedures used to describe "normal". Finally, borderline "normal" values should be interpreted with caution. Such interpretations should, when possible, use additional clinical information in the decisions in order to designate an adverse-effect level or a no-adverse-effect level.

## E.5.1 Interpretive Strategies for Animal Testing

Again, the concepts outlined above generally apply to animal testing with a few notable differences. Although spirometric measures in animals appear to be consistent over time, no real investigation of this has been conducted. It should be pointed out, however, that most rodents grow throughout life and their age dependent spirometry appears to improve (by anthropomorphic standards) over the same period until just before death. This is quite unlike the huan which begins to have less than optimal performance beyond young adulthood

(around 21 years of age). The $DL_{CO}$ in rodents also improves over most of life but begins to diminish before the fall in spirometry. This is not the case in humans.

# APPENDIX F

# CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL LABORATORY ANIMAL TOXICITY STUDIES[1]

A minimally acceptable study should meet the following criteria, which fundamentally represent good scientific practice.

1. All elements of exposure should be clearly defined.

   - The exposure concentration, administration route, exposure schedule, and exposure duration must be described. Consideration should also be given to the concentration and time of exposure used versus the expected level of human exposure.

   - If animal body weights, ages, or sex are not provided, consideration should be given to the uncertainty in appropriate default values.

   - Exposure information should include physicochemical characteristics of the substance used, such as purity, stability, pH, partition coefficient, particle size and distribution, breathing zone concentration, and vehicle. These properties can influence the local effects and the rate and extent of absorption, which can subsequently modify the toxic manifestations. Concentrations should be reported as means and variances.

   - Exposure information should include a description of generation and characterization technology used (e.g., chamber design, type, dimensions, uniformity of distribution, source of air, generating system, air conditioning, and exhaust treatment). The number of air changes, air flow rate, oxygen content, temperature, and relative humidity are exposure chamber characteristics that should be monitored and reported as means and variances. The description of the characterization method(s) should also include frequency of measurement, calibration of the measurement instrument, frequency of the calibration, and other quality assurance elements. Cage (or other animal holder) rotation schedule should be described.

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[1]Adapted from Society of Toxicology (1982), Muller et al. (1984), National Research Council (1984), James (1985), and Lu (1985a).