TOXICOLOGICAL SCIENCES 43, 213–229 (1998)
ARTICLE NO. TX982430

Non-Cancer Risk Assessment for Nickel Compounds: Issues Associated with Dose–Response Modeling of Inhalation and Oral Exposures[1,2]

Lynne T. Haber,* Bruce C. Allen,† and Carole A. Kimmel‡

*ICF Consulting Group, ICF Kaiser International, Fairfax, Virginia 22043; †RAS Associates, Chapel Hill, North Carolina 27514 and ‡National Center for Environmental Assessment, U.S. EPA, Washington, DC 20460

Received June 16, 1997; accepted January 13, 1998

Non-Cancer Risk Assessment for Nickel Compounds: Issues Associated with Dose–Response Modeling of Inhalation and Oral Exposures. Haber, L. T., Allen, B. C., and Kimmel, C. A. (1998). *Toxicol. Sci.* 43, 213–229.

This report presents the results of noncancer dose–response modeling for inhalation and oral exposures to nickel compounds using the NOAEL/LOAEL and benchmark dose (BMD) approaches. Several key issues associated with the implementation of the BMD approach were examined. Primary among them are difficulties associated with use of data for which the dose–response shape is poorly defined: nonuniqueness of maximum likelihood estimates and lower bounds equal to zero. In addition, several generalizable properties of the "hybrid approach" for modeling continuous endpoints were identified. A hybrid modeling approach allows one to consider "biological significance" on an individual (rather than group) basis; differences between individual- and group-based biological significance in the definition of benchmark response (BMR) levels are elucidated. In particular, it is shown that BMDs defined using group-based BMRs may be more like LOAELs than NOAELs. Application of cross-chemical and cross-endpoint comparisons suggest that, for chronic inhalation exposure, nickel sulfate appears to be as toxic or more toxic than nickel subsulfide and nickel oxide, although the high response rates for the latter two compounds at the lowest chronically administered concentration make such conclusions problematic. A nickel reference concentration could be derived based on the most sensitive benchmark concentration for chronic inhalation exposure to nickel sulfate, 1.7×10^{-3} mg Ni/m^3 for lung fibrosis in male rats. Analyses of oral studies of nickel sulfate and nickel chloride suggest that an appropriate basis for the nickel oral reference dose would be a BMD of 4–5 mg Ni/kg/day, based on increased prenatal mortality. (Uncertainty factors were not determined and neither an RfD nor an RfC was derived in this paper.) The BMD approach provides appropriate quantitative support for toxicological judgment; this paper addresses specific issues associated with the role of the BMD approach in noncancer risk assessment. Resolution of these and other issues may require the accumulation of a number of case studies such as the one presented here. © 1998 Society of Toxicology.

Nickel exposure occurs occupationally, primarily via the inhalation route, and through contamination of ambient air, most often as nickel oxide and nickel sulfate. Oral exposure of the general public to nickel is primarily in food, but may also occur via contaminated water (NTP, 1996a,b,c). Nickel is one of the most frequently occurring chemicals in waste sites in the United States. Differences in the inhalation toxicity of the different nickel compounds correlate with differences in their solubility (Dunnick *et al.*, 1988), presumably due to differences in the cellular absorption of the deposited material. The respiratory tract is the primary target of inhaled nickel compounds, indicating substantial portal-of-entry effects (Dunnick *et al.*, 1988, 1989; Benson *et al.*, 1987, 1988, 1990; Lovelace Inhalation Toxicology Research Institute, 1986a,b; NTP, 1996a,b,c). Due to the relative size of cross-species dosimetric adjustments for respiratory and extrarespiratory effects (USEPA, 1994), systemic effects reported by Benson *et al.* (1987, 1988) and Dunnick *et al.* (1988) occur at higher human equivalent concentrations than those inducing respiratory effects.

Subchronic, chronic, reproductive, and developmental studies of oral exposure to nickel compounds are available. In contrast to the inhalation route, ingested soluble nickel compounds are dissociated in the stomach. Therefore, the toxicity of different soluble nickel compounds should depend primarily on the amount of nickel absorbed. The two compounds evaluated in oral studies (nickel chloride and nickel sulfate) have similar water solubilities. Decreased body weight was the most sensitive endpoint in the subchronic study (American Biogenics Corporation, 1988) and the chronic study (Ambrose *et al.*, 1976) investigating general systemic effects. One subchronic study investigated the effects of nickel on the immune system (Dieter *et al.*, 1988). Reproductive and developmental toxicity studies [Smith *et al.* (1993) and Research Triangle Institute (1988), respectively] reported decreased fetal and pup viability.

[1] The work described in this article was supported by the U.S. Environmental Protection Agency (under Contract 68-D2-0129). The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[2] Presented in part at the annual meeting of the Society for Risk Analysis, Washington, DC, December, 1997.

1096-6080/98 $25.00

Ambrose *et al.* (1976) also described a three-generation reproductive study in rats. Schroeder and Mitchener (1971) conducted a three-generation study of rats administered nickel at 5 ppm (estimated at 0.43 mg Ni/kg/day), and observed significantly increased neonatal mortality and incidence of runts. However, this study is limited by the small sample size and by dietary insufficiency of other trace elements, particularly chromium, which may have contributed to toxicity of nickel (IRIS, 1997).

The benchmark dose/benchmark concentration (BMD/BMC) approach has been proposed as an alternative to the NOAEL/LOAEL method for noncancer risk assessment (Crump, 1984). Advantages of the BMD approach over the NOAEL/LOAEL method have been documented in several publications (Crump, 1984; Kimmel and Gaylor, 1988; Barnes *et al.*, 1995), and include reduced dependency on dose selection and dose spacing, more appropriate reflection of sample size, and better inclusion of dose–response information. US EPA guidelines for risk assessment of developmental toxicity (USEPA, 1991) and reproductive toxicity (USEPA, 1996a), guidelines for development of inhalation reference concentrations (RfCs) (USEPA, 1994), and proposed guidelines for neurotoxicity risk assessment (USEPA, 1995) have addressed the application of BMD methodology. Indeed, several reference doses (RfDs) and reference concentrations (RfCs) listed on EPA's Integrated Risk Information System (IRIS) have been developed using BMD methodology, including the methylmercury RfD, and the carbon disulfide, antimony trioxide, and 1,1,1,2-tetrafluoroethane (HFC-134a) RfCs (IRIS, 1997).

A few studies have been published describing the benchmark dose modeling of individual chemicals, including 1,2-dibromo-3-chloropropane (DBCP) (Pease *et al.*, 1991), hydrogen fluoride (Alexeeff *et al.*, 1993), chromium (Malsch *et al.*, 1994), trichloroethylene (Haag-Grönlund *et al.*, 1995), and boric acid (Allen *et al.*, 1996). However, few of these studies have described issues related to the choice of endpoints to model or interpretation of the results. The BMD/BMC modeling of inhalation and oral exposure to nickel described in this report is part of a series of chemical-specific case studies, one goal of which is the development of procedures appropriate for noncancer risk assessments using the BMD methodology. Of particular interest for the nickel case study is consideration of rational operational procedures for selecting endpoints for noncancer risk assessment, including BMD/BMC modeling, for comparing the results across endpoints. An additional goal of this work is to investigate the behavior of the hybrid approach described by Gaylor and Slikker (1990) and elaborated by Crump (1995) for modeling changes in the mean response of continuous endpoints, but defining BMDs/BMCs in terms of probability of response. In particular, the hybrid approach was evaluated in the context of considering biological significance on an individual basis. To those ends, this paper discusses modeling of short-term and chronic inhalation and oral exposure to three nickel compounds, nickel subsulfide, nickel sulfate, and nickel oxide, as well as subchronic inhalation exposure to nickel sulfate.

METHODS FOR MODELING

"Dose" and calculation of the HEC. Inhalation exposure was expressed in terms of nickel concentration (mg Ni/m^3) for all three nickel compounds. Adjustment for discontinuous exposure, to derive continuous exposure equivalents, was performed using the equation

$$E_{(ADJ)} = E \times D\ (\text{h}/24\ \text{h}) \times W\ (\text{days}/7\ \text{days}) \quad (1)$$

where E is the experimental exposure level, D is the number of hours exposed in the experiment/24 h, and W is the number of days of experimental exposure/7 days.

Additional dosimetric adjustments were conducted to derive human equivalent concentrations (HECs), using the method of Jarabek *et al.* (1990) and USEPA (1994) to account for differences in particle deposition in different respiratory tract regions of animals and humans. The respiratory tract region affected by the endpoint of interest was identified. The Regional Deposited Dose Ratio (RDDR) was then calculated using the RDDR program (USEPA, 1994), based on the region of interest, animal species, strain, and sex; and the particle characteristics (mass median aerodynamic diameter, MMAD, and geometric standard deviation, σ_g). The HEC is the product of the RDDR and the duration-adjusted exposure concentration. The HEC values, in units of mg Ni/m^3, were used as input to the dose–response models.

Although other methods for interspecies extrapolation of inhalation particle dosimetry are available (e.g., Asgharian *et al.*, 1995) and dosimetric adjustment for exposure to particulates includes several areas of uncertainty, alternative approaches were not investigated here. The focus of this analysis was on issues associated with the application of the BMD approach to a series of related endpoints, rather than on the specific values of BMCs or other estimates, all of which could be affected by the choice of HEC calculation method.

No adjustments were conducted for oral dosing, because all of the modeled oral studies used continuous dosing protocols.

Quantitative response data. The data sets considered for dose–response analysis included both quantal and continuous endpoints. The candidate endpoints needed to have quantitative response data in either case. Quantitative response data for quantal results are summarized in terms of number affected out of total number examined.

Continuous endpoints (e.g., body weights) are those for which "counts" do not typically apply. Quantitative response data for such endpoints are satisfactorily expressed in terms of a mean and a standard deviation (or other measure of variability) for each dose group.

Mathematical dose–response modeling. The quantal endpoints were modeled using the standard Weibull and polynomial models (Crump, 1984). A "threshold" (intercept) parameter was included in the modeling only when a sufficient number of dose groups were available (at least four) and when the models without a threshold provided a relatively poor fit to the data. The degree of the polynomial model was restricted to be no greater than the number of dose groups minus one. The software packages THRESH and THRESHW (ICF Kaiser International, KS Crump Group), which fit the models by methods of maximum likelihood, were used to implement these models.

For the continuous endpoints, we used the "hybrid" modeling approach described by Gaylor and Slikker (1990) and elaborated by Crump (1995). This approach uses all of the information contained in the original observations, by modeling changes in mean response as a function of dose, but defines BMDs/BMCs in terms of probability of response.

For the hybrid modeling approach, the two models used to describe how the probability of response is assumed to vary with dose are the Weibull model and the power model. The Weibull model,

$$P(d) = p_0 + (1 \quad p_0)[1 \quad \exp\{-(\beta * d)^\gamma\}] \quad (2)$$

is identical to the Weibull model used for the quantal endpoints. Maximum likelihood methods are used to estimate the parameters, β and γ, as well as a background mean response level, $m(0)$, and a fixed standard deviation estimate for all dose groups, σ. If a normal distribution for the continuous measure around the dose-specific means is assumed, the Weibull model can be expressed as the change in mean as a function of dose:

$$m(d) = m(0) + \sigma[N^{-1}(1 - p_0) - N^{-1}((1 \quad p_0)\exp\{ \quad [\beta * d)^{\gamma}\})] \quad 3)$$

where N^{-1} is the inverse normal function.

The power model was also used to model continuous endpoints:

$$m(d) = \alpha + (\beta * d)^k \quad (4)$$

where $m(d)$ is the mean response at dose d and the three unknown parameters, α, β, and k, as well as the dose group standard deviations, are estimated by maximum likelihood methods. The dose group standard deviations estimated by the model account both for the variation in the observed data and for any difference between the observed mean and the mean estimated by the model. The parameter k is not constrained to be an integer, but it is constrained to be greater than or equal to 1. The underlying change in probability of response as a function of dose induced by Eq. (4) (assuming normal variation around the dose-specific means and a constant variance) is

$$P(d) = \quad N[N^{-1}(1 \quad p_0) - (\beta * d)^k/\sigma], \quad (5)$$

where N is the cumulative normal function, N^{-1} is its inverse, and σ is the standard deviation assumed for all dose levels. This form is for those cases in which increased values of the endpoint are adverse; a similar equation holds for those cases in which decreased values of the endpoint are considered adverse. The Weibull and power models for continuous endpoints were fit using the software program Bench_C (ICF Kaiser International, KS Crump Group).

Use of the Weibull or power models for continuous endpoints requires definition of a background incidence of abnormality, p_0, or the specification of a level of response that can be considered the cut-point between normal and abnormal responses, x_0. Specification of p_0 (and of the type of distribution—assumed here to be normal for all endpoints) implicitly defines a cut-point, x_0, when the parameters for the background variability are estimated as part of the modeling. Similarly, specification of a cut-point determines the background incidence once the background variability is estimated (Crump, 1995). The BMD is then defined as the lower bound on dose at which the increased probability of an abnormal response is equal to 10% (see below). In the absence of endpoint-specific toxicology data to support a choice of a p_0 or an x_0 value, we examined a range of p_0 values (0.001, 0.01, 0.05) to evaluate the sensitivity of the predicted BMD to the selection of p_0. The cut-point, x_0, was specified only in the case of models applied to body weights [data from Ambrose *et al.* (1976), and American Biogenics Corporation, (1988)].

The continuous form of the Weibull model used here assumes that the standard deviation is constant for all dose groups. The power model was run either assuming a constant variance or allowing dose-specific standard deviations. Although the standard deviations do not appear explicitly in the power model [Eq. (4)], they are also estimated and affect estimates of the probability of response [see Eq. (5)]. In the Research Triangle Institute (1988) study, the study authors did not state whether the reported measures of variability were standard deviations (SDs) or standard errors (SEs); SEs were assumed for the modeling.

The benchmark response (BMR) considered in these analyses was 10% extra risk. That is, the BMD for any particular combination of endpoint, model, p_0, etc., is defined as the lower bound on the dose, d_m, for which

$$P(0)]/[1 \quad P(0)] = 0.10 \quad (6)$$

The dose d_m itself is referred to as the maximum likelihood estimate (MLE) of the dose corresponding to the specified level of risk. In other contexts it may be referred to as the ED_{10}.[3]

In order to mimic a commonly accepted practice with respect to body weight, the power model [Eq. (4)] and a corresponding continuous polynomial model,

$$m(d) = \alpha + \beta_1 d + \beta_2 d^2 + \quad + \beta_k d^k \quad (7)$$

were applied to the body weight endpoints and used to predict the doses for which there would be a 10% change in mean weight. This approach was implemented using the THC and THWC programs (ICF Kaiser International, KS Crump Group). The BMDs derived in this approach do not correspond to specified changes in the probability of response. Rather, they correspond to doses for which the relative change in mean response ($[m(d) - m(0)]/m(0)$) is 0.10, regardless of variability around the means. Moreover, because individual body weight data were available from one study (American Biogenics Corporation, 1988), the body weights were quantalized (considered abnormal if they were below a chosen x_0 and normal otherwise), and the counts of abnormal body weights were modeled using the quantal Weibull and polynomial models.

For the quantal models, goodness of fit was determined using the χ^2 test. For the continuous models, goodness of fit was determined using an F test that normalizes the differences between the observed and predicted means (using variabilities observed within dose groups) and accounts for the degrees of freedom associated with the predictions and those associated with the within-group variability.

A likelihood ratio test was performed to determine if consideration of dose-specific standard deviations significantly improved the fit of the power model to the continuous endpoints. When no significant improvement was found ($p > 0.05$), only results corresponding to the power model with constant variance were presented.

RESULTS AND DISCUSSION

Choice of Studies and Endpoints to Model

Studies. Studies were selected for modeling based on the quality of the study design, the biological significance of the endpoints observed, and the suitability of the data presentation for modeling. At least three exposure groups were needed, to better establish the shape of the dose–response curve. The study report needed to adequately define the protocol, including the nickel compound studied, the dosing or exposure duration, and the size of the dose groups. Quantitative response data (see Methods for Modeling) were also necessary. For inhalation studies, the MMAD and σ_g were also required for dosimetric adjustments (Jarabek *et al.*, 1990; USEPA, 1994).

The inhalation studies satisfying these criteria constitute a series of related reports of the effects in male and female F344/N rats and B6C3F1 mice of inhalation exposure to nickel subsulfide, nickel sulfate, and nickel oxide for subacute, subchronic, and chronic durations. These studies include Dunnick

[3] For the continuous endpoints, doses corresponding to 10% additional risk rather than 10% extra risk were calculated. Because the assumed background rates were relatively low (less than 5% when estimated directly and less than 14% when determined from the specification of a normal/abnormal cut-point, x_0) there will be very little difference between extra and additional risk for these endpoints, and thus little difference between the BMD estimates derived here and those corresponding to 10% extra risk.

et al. (1988, 1989), Benson *et al.* (1987, 1988, 1990), Lovelace Inhalation Toxicology Research Institute (1986a,b), and NTP (1996a,b,c). Exposures were conducted for 6 h/day, 5 days/week, for 16 days (12 exposures) in the subacute studies, for 13 weeks in the subchronic studies, and for 2 years in the chronic studies. Because of slight differences in the reported response rates among these studies, the NTP data were considered for modeling because more detailed information on the responses and particle characterization was provided in the NTP reports. The MMAD for all of the NTP studies was in the range 1.9 to 3.0, and the σ_g ranged from 1.9 to 2.4. Respiratory effects were the most sensitive endpoints. Mice were less sensitive than rats to respiratory effects of inhaled nickel compounds, although they were more sensitive to the lethal effects of nickel subsulfide and nickel sulfate (NTP, 1996a,b). This paper concentrates on the more sensitive species, and therefore does not discuss results in mice.

The oral studies satisfying the criteria for modeling include two subchronic studies (American Biogenics Corporation, 1988; Dieter *et al.*, 1988) and one chronic study (Ambrose *et al.*, 1976). While Dieter *et al.* (1988) focused on immune system responses, the other two investigations looked for general systemic effects. In addition, two reproductive toxicity studies (Research Triangle Institute, 1988; Smith *et al.*, 1993) were considered adequate for analysis.

Endpoints. The biological significance and adversity of the endpoints in the selected studies is an important consideration. As an example, for the three compounds under consideration, all the inhalation exposures for most of the exposure durations induced alveolar macrophage hyperplasia (at 100% incidence at the low concentration in at least one species/sex in many of the studies). Although this is a sensitive endpoint, it was considered to be "pre-adverse," as it consisted of a slight increase in the number of alveolar macrophages and was described as only subtly different from controls. Such slight increases in macrophage number can also be seen with nuisance dusts, although the low exposure concentration here and the differences observed with nickel exposure between rats and mice indicate that the effect is compound-related. Nevertheless, because this effect was considered "pre-adverse," it was not modeled.

The final step in choosing endpoints appropriate for modeling is to identify the more sensitive of the biologically relevant endpoints for each exposure duration and nickel compound. For inhalation experiments, that determination is made after adjustments for intermittent exposure and after calculation of the HECs (see Methods for Modeling), in order not to miss the endpoints that would apparently be more sensitive in humans exposed continuously. The endpoint(s) with the lowest NOAEL(HEC) and/or LOAEL(HEC) were selected for modeling. In addition, the magnitude of the response (especially for quantal endpoints) is considered in choosing the most sensitive endpoints.

Modeling of Inhalation Studies

Table 1 lists the most sensitive quantal and continuous endpoints from the inhalation studies of nickel compounds. When one sex was clearly more sensitive, due to a higher quantal response at the same HEC or a lower HEC for the same response level, only the results for the more sensitive sex are presented.

Subacute subsulfide. Acceptable fits were obtained for the modeling of olfactory epithelial atrophy of male and female rats exposed to nickel subsulfide, with a slightly lower BMC calculated for males (Fig. 1, Table 2). The lung inflammation endpoint (see Table 1) was modeled for the information it provided on the upper bounds, rather than to obtain the MLE or BMC. Because lung inflammation was observed in 100% of the males and females at all positive exposure levels, no unique maximum likelihood estimates exist (e.g., in the Weibull model, the parameters α, β, and γ could not be uniquely estimated). The lower bound on concentration corresponding to any BMR for this endpoint is 0; the upper bound on the concentration corresponding to 10% risk of lung inflammation was 0.034 mg Ni/m^3 (Weibull model) and 0.016 mg Ni/m^3 (polynomial model) for both males and females. The significance of the upper bound for comparisons among the different nickel compounds is addressed below.

Subacute sulfate. A 100% response was observed for olfactory epithelial atrophy and lung inflammation in females and males following subacute exposure to nickel sulfate. Because the HEC values for males were higher than those for females for the same exposure levels and the response was the same, the results from males are not presented. For both of these endpoints, the BMCs determined from such minimal data sets would be zero. The upper bounds on concentration corresponding to 10% risk for the females were 0.011 mg Ni/m^3 (Weibull model) and 0.0054 mg Ni/m^3 (polynomial model) for the nasal endpoint. For the lung endpoint, the upper bounds for females were 0.066 mg Ni/m^3 (Weibull model) and 0.032 mg Ni/m^3 (polynomial model). Different upper bounds were obtained for the two endpoints due to the different dosimetric adjustments for the extrathoracic and pulmonary regions.

Subacute oxide. The concentration–response curve for alveolar inflammation following subacute exposure to nickel oxide was also quite steep, increasing from 0% at 3.9 mg Ni/m^3 to 100% at 7.9 mg Ni/m^3 (Table 1). Although the alveolar inflammation response jumped from 0 to 100%, a BMC can be calculated for this endpoint, because there were positive exposure levels below the level that induced a 100% response. Interestingly, the BMC is less than the highest concentration for which 0% response was observed (0/5 responders observed at the NOAEL[HEC] of 0.42 mg Ni/m^3, versus a BMC[HEC] of 0.34 mg Ni/m^3). This result is consistent with the fact that the observation of 0 responders out of 5 animals on test is in no way conclusive evidence that no risk exists at that exposure level. In fact, if the risk (probability of response) at that level of exposure were 0.10, one would expect to observe a response rate of 0/5 almost

TABLE 1
Selected Nickel Inhalation Data Considered for Modeling[a]

Duration/nickel compound	Exposure level (mg Ni/m^3)	Effect observed	Response	Region[b]	NOAEL/LOAEL; NOAEL(HEC)/ LOAEL(HEC) (mg/m^3)
Subacute/subsulfide	0, 0.44, 0.88, 1.8, 3.6, 7.3	Atrophy of olfactory epithelium in males 0, 0.012, 0.023, 0.045, 0.091, 0.19	0/5, 4/5, 4/5, 5/5, 5/5, 5/5	ET	None/0.44[c]; None/0.012[c]
		Atrophy of olfactory epithelium in females 0, 0.0076, 0.015, 0.029, 0.059, 0.12	0/5, 2/5, 5/5, 5/5 5/5, 5/5	ET	0.44/0.88; 0.0076/0.015
		Lung inflammation in males 0, 0.039, 0.078, 0.16, 0.32, 0.65	0/5, 5/5, 5/5, 5/5, 5/5, 5/5	PU	None/0.44[c]; None/0.039[c]
		Lung inflammation in females 0, 0.039, 0.078, 0.16, 0.32, 0.65	0/5, 5/5, 5/5, 5/5 5/5, 5/5	PU	None/0.44[c]; None/0.039[c]
Subacute/sulfate	0, 0.8, 1.6, 3.3, 6.7, 13.3	Atrophy of olfactory nasal epithelium in females 0, 0.013, 0.026, 0.053, 0.11, 0.21	0/5, 5/5, 5/5, 5/5 5/5, 5/5	ET	None/0.8[c]; None/0.013[c]
		Lung inflammation in females 0, 0.077, 0.15, 0.32, 0.64, 1.3	0/5, 5/5, 5/5, 5/5, 5/5, 5/5	PU	None/0.8[c]; None/0.077[c]
Subacute/oxide	0, 0.9, 2.0, 3.9, 7.9, 23.6	Alveolar inflammation of the lung in females 0, 0.097, 0.22, 0.42, 0.85, 2.5	0/5, 0/5, 0/5, 0/5, 5/5, 5/5	PU	3.9/7.9 0.42/0.85
		Lung weight in females (as reported by Dunnick *et al.* 1988)	Continuous	TH	3.9/7.9 0.41/0.83
Subchronic/sulfate	0, 0.027, 0.056, 0.11 0.22, 0.45	Olfactory epithelial atrophy in females 0, 0.00044, 0.00084, 0.0016, 0.0036, 0.0075	0/10, 0/10, 1/10, 2/10, 10/10, 10/10	ET	0.11/0.22; 0.0016/0.0036
Chronic/subsulfide	0, 0.11, 0.73	Chronic active inflammation of lung in males 0, 0.0090 (high group dropped)	9/53, 53/53, 51/53	PU	None/0.11[c]; None/0.0090[c]
		Lung fibrosis in males 0, 0.0090, 0.065	2/53, 48/53, 40/53	PU	None/0.11[c]; None/0.0090[c]
		Lung fibrosis in females 0, 0.010, 0.074	0/53, 50/53, 44/53	PU	None/0.11[c]; None/0.010[c]
		Proteinosis of alveolus in females 0, 0.010, 0.074	2/53, 49/53, 53/53	PU	None/0.11[c]; None/0.010[c]
		Atrophy of olfactory epithelium in females 0, 0.0036, 0.027	0/53, 0/53, 16/52	ET	0.11/0.73; 0.0036/0.027
Chronic/sulfate	0, 0.027, 0.056, 0.11	Lung fibrosis in males 0, 0.0021, 0.0046, 0.0095	3/54, 6/53, 35/53, 43/53	PU	0.027/0.056; 0.0021/0.0046
		Lung fibrosis in females 0, 0.0024, 0.0052, 0.011	8/52, 7/53, 45/53, 49/54	PU	0.027/0.056; 0.0024/0.0052
		Alveolar proteinosis in females 0, 0.0024, 0.0052, 0.011	1/52, 0/53, 22/53, 49/54	PU	0.027/0.056; 0.0024/0.0052
		Atrophy of olfactory epithelium in females 0, 0.00084, 0.0019, 0.0039	0/51, 1/52, 1/53, 7/54	ET	0.056/0.11; 0.0019/0.0039
Chronic/oxide	0, 0.49, 0.98, 1.96	Chronic lung inflammation in males 0, 0.042 (two high groups dropped)	28/54, 53/53, 53/53, 52/52	PU	None/0.49[c]; None/0.042[c]
		Chronic lung inflammation in females 0, 0.049, 0.10, 0.21	18/53, 52/53, 53/53, 54/54	PU	None/0.49[c]; None/0.049[c]

[a] From NTP (1996a,b,c), unless otherwise noted.
[b] Respiratory tract regions: ET, extrathoracic; PU, pulmonary; TH, thoracic.
[c] Concentration shown is the lowest tested concentration. These are not true LOAELs, in light of the high response.

60% of the time. The BMC, which is represented by a confidence limit, correctly reflects that possibility.

Nickel oxide also induced a gradual increase in lung weight at all exposure levels (Dunnick *et al.*, 1988), becoming significant in a pairwise test at 7.9 mg Ni/m^3. This continuous endpoint was modeled using the "hybrid approach," with the BMD defined in terms of a probability of response for this continuous endpoint (Crump, 1995). Because little information is available on the modeling conditions that correspond best to NOAELs, several different combinations were explored. Thus, BMRs of 5 and 10% were determined, using values of 0.001, 0.01, and 0.05 for the background incidence of a lung weight

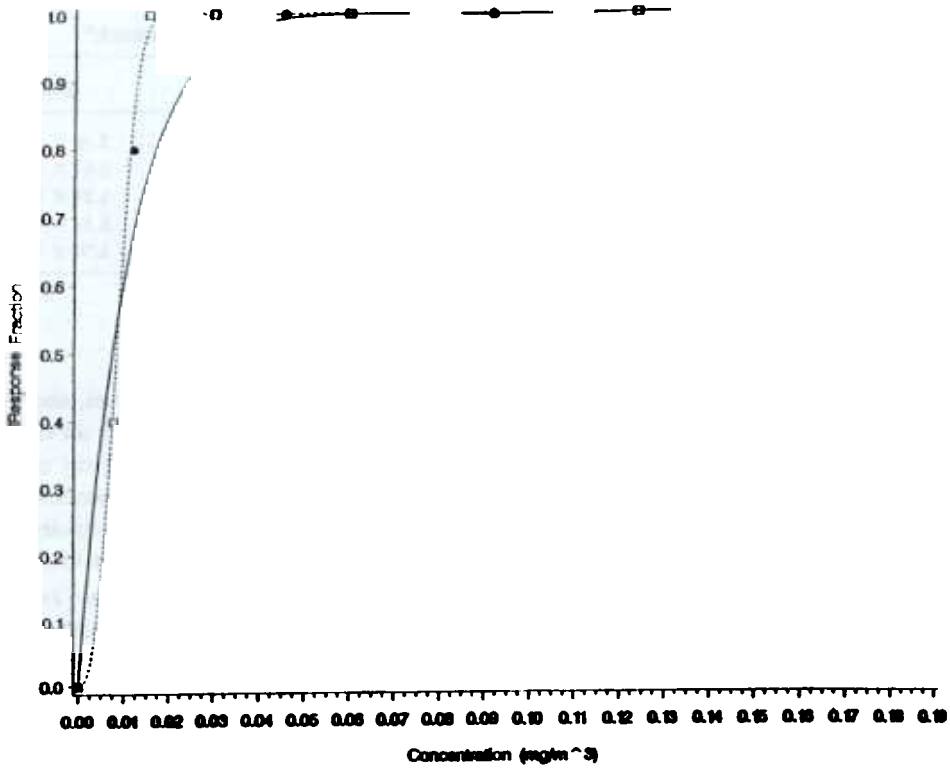

FIG. 1. Graphical display of the results of benchmark modeling using the polynomial model for atrophy of the olfactory epithelium exposed to nickel subsulfide for a subacute duration. Data points (and MLE lines) are shown as filled circles (—) for male rats, and squares (- - -) for females rats.

that would be considered an adverse effect (p_0). Trends and interpretations of the various combinations of BMR and p_0 values are addressed below. Modeling was done using the Weibull and power models (Table 3, Fig. 2).

Subchronic sulfate. Modeling was conducted for the subchronic studies with all three nickel compounds, but only data related to the subchronic study with nickel sulfate are presented (Table 4). Of the three compounds tested, nickel sulfate was the most toxic following subchronic exposure (data not shown). Only one endpoint, olfactory epithelial atrophy in female rats, was modeled for the subchronic study of nickel sulfate. The fit was excellent ($p = 0.96$), and the BMC was $4.8E^{-4}$ mg Ni/m^3 (Fig. 3). Uncertainty in the modeling is relatively low, due to the presence of three nonzero data points in the low-exposure region (Table 1).

Chronic subsulfide. Because there were only high rates of response for most of the effects observed after chronic exposure to nickel subsulfide, the only modeling pursued was for olfactory epithelial atrophy. An excellent fit was obtained (Table 2), and the BMC(HEC) for that endpoint (0.01 mg Ni/m^3) was similar to the HECs corresponding to high response levels (91 to 100%) for the other endpoints (0.009 to 0.01 mg Ni/m^3; see Table 1). That is, the lower bound on dose corresponding to a 10% risk of olfactory epithelial atrophy is nearly the same as the dose observed to yield high rates of lung effects. Not surprisingly, these modeling results do not alter the impression that the most sensitive endpoints were chronic active lung inflammation and lung fibrosis.

Chronic sulfate. In contrast to the data for most of the other nickel compounds and durations, the exposure levels tested for

TABLE 2
Quantal Results for Nickel Subsulfide Inhalation[a]

Exposure duration	Endpoint	Polynomial			Weibull		
		MLE	BMC	G-O-F p value	MLE	BMC	G-O-F p value
Subacute	Atrophy of olfactory epithelium in male rats	1.10×10^{-3}	5.95×10^{-4}	8.46×10^{-1}	1.07×10^{-3}	5.93×10^{-4}	9.35×10^{-1}
Subacute	Atrophy of olfactory epithelium in female rats	4.04×10^{-3}	6.20×10^{-4}	9.38×10^{-1}	4.08×10^{-3}	6.76×10^{-4}	9.86×10^{-1}
Chronic	Atrophy of olfactory epithelium in female rats	1.46×10^{-2}	1.02×10^{-2}	8.40×10^{-1}	2.48×10^{-2}	1.03×10^{-2}	1.00×10^{0}

[a] All modeled data from NTP (1996a), BMR of 10%.

TABLE 3
Results of Modeling Increased Lung Weight Using the Hybrid Approach[a]

Model	P0	X0	BMR	MLE	BMD	G-O-F *p* value
Weibull, P0 fixed	5.00×10^{-2}	1.03×10^{0}	1.00×10^{-1}	2.70×10^{-1}	7.48×10^{-2}	4.88×10^{-1}
Weibull, P0 fixed	5.00×10^{-2}	1.03×10^{0}	5.00×10^{-2}	1.44×10^{-1}	3.64×10^{-2}	4.88×10^{-1}
Weibull, P0 fixed	1.00×10^{-2}	1.13×10^{0}	1.00×10^{-1}	6.53×10^{-1}	1.21×10^{-1}	4.79×10^{-1}
Weibull, P0 fixed	1.00×10^{-1}	1.25×10^{0}	1.00×10^{-1}	1.66×10^{0}	3.31×10^{-1}	4.67×10^{-1}
K Power, P0 fixed	5.00×10^{-2}	1.07×10^{0}	1.00×10^{-1}	6.86×10^{-1}	4.56×10^{-1}	5.35×10^{-1}

[a] Data from Dunnick *et al.* (1988)

nickel sulfate provided useful information on the shape of the concentration–response curve in the low-concentration region (Table 1). The modeling results for those endpoints (Table 4) show that inclusion of a "threshold" (intercept) parameter in the models (and the restriction of the power parameter to its limiting value of 1) improved the fits in many cases and was required in order to obtain adequate or marginal fits in some cases (see Fig. 4). Note that the threshold parameter is purely a statistical parameter, and is not necessarily related to a true biological threshold. The threshold parameter bounds the region of the dose–response curve where the model estimates the same response as background for the observed effect.

Chronic oxide. All of the endpoints induced by chronic exposure to nickel oxide reached or neared 100% response at the lowest positive exposure level, and there was a high background incidence of response for all endpoints. The BMCs for such endpoints are subject to great uncertainty because of a lack of information about the concentration–response behavior, and would be equal to zero for endpoints with a 100% response at all nickel oxide concentrations tested.

Information on dose–response relationships. There are several related well-conducted studies addressing the inhalation toxicity of subacute, subchronic, and chronic exposure to nickel subsulfide, nickel sulfate, and nickel oxide. As shown above, however, there was little or no information on the shape of the exposure–response curve for many of the most sensitive endpoints from these studies. For many of the endpoints, the lowest concentration gave a response incidence at or near 100%. Thus, for

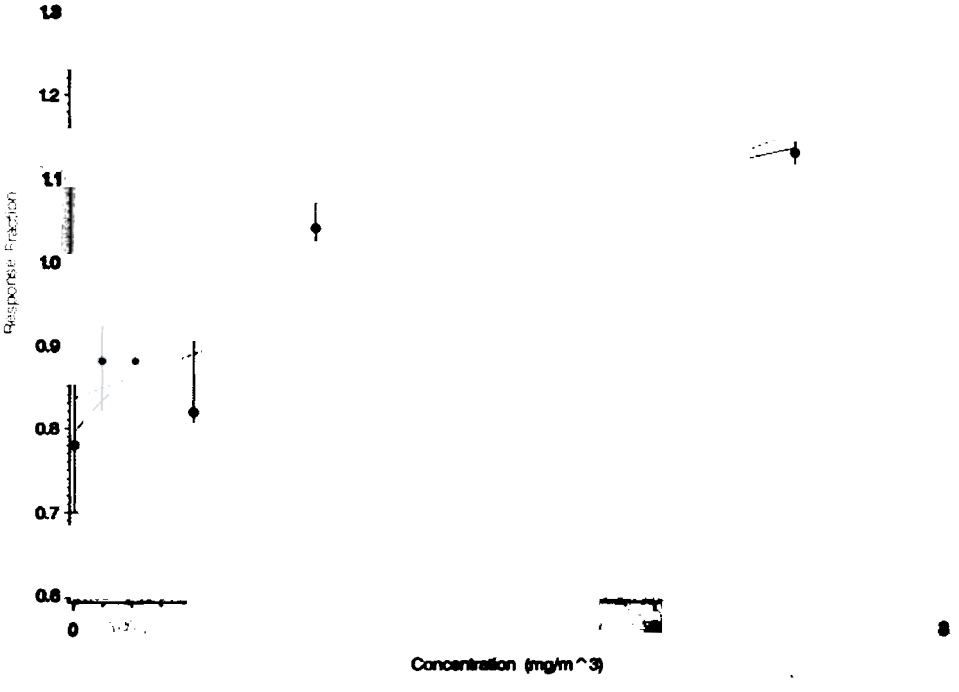

FIG. 2. Graphical display of the results of benchmark modeling using the hybrid approach for increased lung weight in females exposed to nickel oxide for a subacute duration. Data points represent the mean ± standard deviation. (—), Weibull model, (- - -), power model.

TABLE 4
Quantal Results for Nickel Sulfate Inhalation[a]

Duration	Endpoint	Compute threshold	Polynomial MLE	Polynomial BMC	Polynomial G-O-F *p* value	Weibull MLE	Weibull BMC	Weibull G-O-F *p* value
Subchronic	Olfactory epithelial atrophy in female rats	Yes	9.84×10^{-4}	5.30×10^{-4}	9.85×10^{-1}	1.12×10^{-3}	7.25×10^{-4}	7.17×10^{-1}
		No	1.10×10^{-3}	4.78×10^{-4}	9.59×10^{-1}	1.12×10^{-3}	7.25×10^{-4}	7.17×10^{-1}
Chronic	Lung fibrosis in male rats	Yes	2.22×10^{-3}	1.70×10^{-3}	3.21×10^{-2}	2.22×10^{-3}	1.70×10^{-3}	3.21×10^{-2}
		No	1.22×10^{-3}	6.87×10^{-4}	7.53×10^{-4}	1.38×10^{-3}	8.35×10^{-4}	1.65×10^{-3}
Chronic	Lung fibrosis in female rats	Yes	2.69×10^{-3}	2.31×10^{-3}	1.06×10^{-3}	2.69×10^{-3}	2.31×10^{-3}	1.06×10^{-3}
		No	1.64×10^{-3}	7.75×10^{-4}	0.00	1.46×10^{-3}	9.29×10^{-4}	0.00
	Alveolar proteinosis in female rats	Yes	3.15×10^{-3}	2.82×10^{-3}	1.00×10^{0}	3.60×10^{-3}	2.83×10^{-3}	1.00×10^{0}
		No	3.27×10^{-3}	2.47×10^{-3}	1.04×10^{-2}	3.21×10^{-3}	2.55×10^{-3}	1.62×10^{-2}
	Atrophy of olfactory epithelium in female rats	Yes	3.50×10^{-3}	2.60×10^{-3}	7.33×10^{-1}	3.45×10^{-3}	2.50×10^{-3}	3.21×10^{-1}
		No	3.50×10^{-3}	2.60×10^{-3}	7.33×10^{-1}	3.45×10^{-3}	2.50×10^{-3}	3.21×10^{-1}

[a] All modeled data from NTP (1996a), BMR of 10%.

example, an extrapolation from 80% response in the study to 10% response (i.e., 10% extra risk) was required for olfactory epithelial atrophy in male rats following subacute nickel subsulfide exposure. One advantage of the BMD methodology is that it can be used to identify a BMD when a study does not identify a NOAEL (Crump, 1984; Allen *et al.*, 1996). Nevertheless, large uncertainties are associated with data sets lacking information on the shape of the dose–response curve in the region where response rates are similar to the BMR of interest. This may be one reason Barnes *et al.* (1995) cautioned against extrapolating from high to low response rates. In this case, the lowest positive exposure concentration differed from the best estimate of the concentration associated with 10% response (the MLE) by more than a factor of 10 (Fig. 1, Table 2).

Such uncertainties contribute directly to the size of the confidence limits around best estimates. The extreme examples are those instances (e.g., lung inflammation after subacute exposure to nickel subsulfide) where 100% of the animals

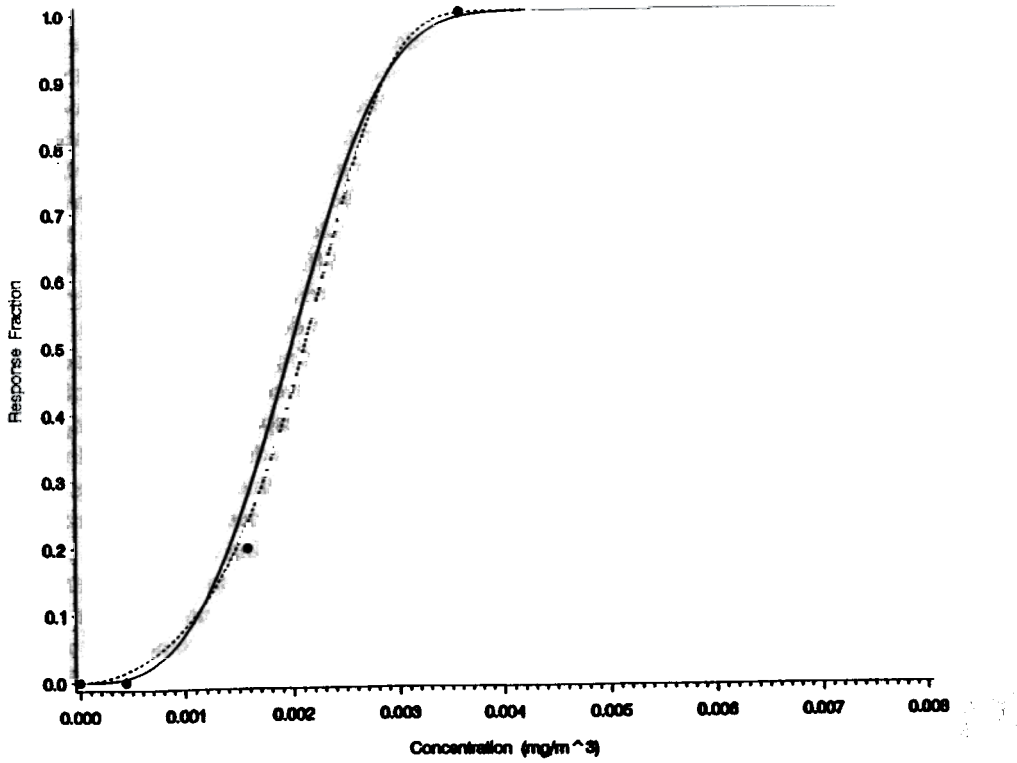

FIG. 3. Graphical display of the results of benchmark modeling using the Weibull model (—) and polynomial model (- - -) for olfactory epithelial atrophy in female rats exposed to nickel sulfate for a subchronic duration.

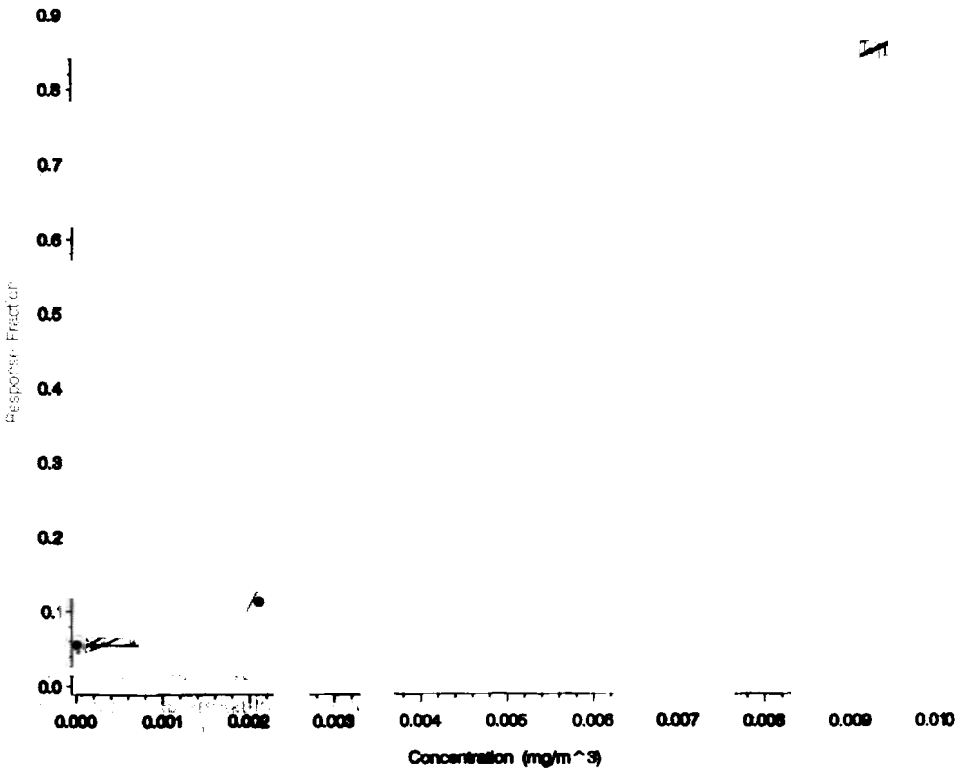

FIG. 4. Graphical display of the results of benchmark modeling for lung fibrosis in male rats exposed to nickel sulfate for a chronic duration. (—), Modeling with the threshold calculated by the program (identical results for the polynomial and Weibull models); (– – –), modeling with threshold set to 0, polynomial model; (– – – –) modeling with threshold set to 0, Weibull model.

responded at the lowest positive dose. In those cases, there is absolutely no information on the shape of the dose–response curve between 0 response and 100% response. As a consequence, unique maximum likelihood estimates do not exist, and the lower confidence limit on the dose corresponding to any response level is 0. The statistical methodology is consistent with the toxicological uncertainty; the dose associated with any particular response level is unknown.

Note, however, that the level of response at the lowest positive dose is not the only, and perhaps not the major, factor determining the size of the confidence limits. In the case of atrophy of the olfactory epithelium following subacute exposure of female rats to nickel subsulfide, the response at the lowest positive dose was 40%, half the response rate observed for the same endpoint in male rats (see Table 1). For the females, the MLE for a 10% response was less than a factor of two below the lowest exposure level (the LOAEL[HEC]), and the BMC was about a factor of 6.5 less than the MLE. For males, the MLE was more than a factor of 10 less than the LOAEL(HEC), but there was only a 1.8-fold difference between the MLE and the BMC. The reason for the difference in the ratios between the MLE and BMC for these two data sets is that the best estimate (i.e., the MLE) of the shape of the dose–response curve for females was nonlinear, while the MLE curve was linear for males. By contrast, linearity could not be ruled out in either case, leading to a linear lower bound (related to the BMC estimate) for both sexes. The difference between the nonlinear best estimate and the linear lower bound (as observed for females) is associated with a larger MLE: BMC ratio than if the best estimate and the lower bound had both been based on linear concentration–responses. Thus, although the large response at the lowest positive dose observed for the males might be expected to lead to large uncertainty (i.e., big differences between the MLE and the lower bound), larger differences can appropriately arise simply because of the shape of the dose–response curve.

An interesting contrast is provided by the data for subacute nickel oxide alveolar inflammation of the lung in females. For that endpoint, the response rate also jumped from 0 to 100% with no observed intermediate responses. However, that increase did not occur until the fifth of six exposure groups. As in all the cases with no observed intermediate response rates, the MLE of the concentration corresponding to 10% extra risk is not unique. Nevertheless, a lower bound on that concentration can be calculated (for any particular model) and that lower bound is not zero. It is lower than the highest concentration for which 0% response was observed (0.34 mg Ni/m^3 vs the NOAEL(HEC) of 0.42 mg Ni/m^3) because of the uncertainty associated with the relatively small, but not necessarily zero, probability of response at that NOAEL-(HEC). Particularly in light of the small sample size, the data set available is not sufficient to rule out 10% response rates below the NOAEL(HEC).

This lower bound might be meaningful for some risk assessment purposes. For example, the lower bound for the most sensitive subacute nickel oxide endpoint is larger than the upper bound for all of the most sensitive subacute nickel subsulfide and nickel sulfate endpoints (the largest upper bounds are 0.034 mg and 0.066 mg Ni/m^3 for the subsulfide and sulfate, respectively). In other words, nickel oxide was significantly less toxic than the other two compounds in the subacute study, as determined by a comparison of concentrations corresponding to 10% risk, a finding that should be usable in a comparative risk analysis. Clearly, in this case, where the number of exposure groups and the HEC values themselves are very similar across compounds, a model-based comparison of this nature may not be required to reach consensus on relative rankings of toxicity. However, other situations may occur for which cross-compound differences in dose levels, spacing between doses, and numbers of doses tested make quick-and-dirty NOAEL/LOAEL comparisons unsatisfactory.

The above discussion does not imply that the lower bounds calculated for these endpoints with little or no information about the shape of the dose–response curve can or should be used for all purposes, especially for setting regulatory exposure limits. In fact, the data that are available for subacute exposures cannot identify the most sensitive endpoint for any compound. Thus, whereas smaller dose-level extrapolations (e.g., from 20 or 40% incidence) may be appropriate, extrapolation from higher response levels (e.g., from 80% incidence or greater) to a BMR of 10% should be avoided.

On the other hand, related studies may provide relevant information on the shape of the dose–response curve in some situations, and allow modeling in otherwise problematic instances. For example, the respiratory endpoints for chronic nickel sulfate exposure exhibited a more gradual increase at low concentrations, followed by a steep increase; these data were best fit by models that incorporated a "threshold" parameter. The information on the shape of the concentration–response curve for nickel sulfate could not be incorporated into the modeling for chronic nickel subsulfide or nickel oxide responses, because there were no data related to the size of a relevant "threshold" value for those compounds. It might be appropriate to use knowledge of the similarities of these three compounds to determine if a "threshold" similar to that observed for nickel sulfate would be expected for the other two compounds. If so, then that information could be used in conjunction with the observed concentration–response data for subsulfide and oxide to constrain the allowable concentration–response curve shapes. That was not done here and the best-fitting concentration–response models for chronic subsulfide and oxide exposure all predicted relatively steep concentration–response behaviors starting immediately above zero concentration. These results were considered to be no more useful than the LOAELs.

The concerns raised here about extrapolating from high response levels also apply, at least to some degree, to the NOAEL/LOAEL method. Calculation of an RfC or RfD by any method that extrapolates from a dose that produces 100% response would be problematic, and might be a reason not to derive such a value. If a dose producing 100% response were used as the basis for an RfC or RfD, considerable toxicological judgement would be required to evaluate the associated uncertainties. Information on the severity of the response would be an important factor in assessing this issue. As for the benchmark approach, knowledge of the shape of the dose–response curve is useful. In this case, the steep exposure–response observed for nickel sulfate may suggest that the response would also decrease rapidly from the high responses observed with the other compounds, and the NOAEL might not be much lower than the lowest concentration tested. Nonetheless, the quality of a risk assessment for nickel subsulfide and nickel oxide would be markedly improved by toxicity data at lower response levels.

Hybrid modeling of continuous endpoints. The continuous endpoint observed after subacute inhalation of nickel oxide was modeled using the "hybrid approach," with the BMD defined in terms of a probability of response. Table 3 illustrates some of the trends and interpretations of the various combinations of BMR and p_0 values relevant to the use of that approach. Primary, and generalizable, conclusions are readily apparent from the results. Illustrative data for the lung weight endpoint are shown in this table; the generalizations described here reflect the characteristics of the model and have been observed for all of the many other endpoints that we have modeled, including those for the oral exposures discussed below.

The BMDs (for fixed BMR) for smaller values of p_0 are greater than those for larger values of p_0. This arises as a result of the shape of the normal distribution assumed for the variability of the responses, the so-called "bell-shaped" curve. The p_0 value specifies how far out in the tail of the distribution the adverse response is: for smaller p_0, fewer unexposed animals are considered to be in the abnormal range. As dose changes, we are basically considering shifts in the distributions; the mean value for the endpoint that is being measured changes to reflect the shift to more adverse effects, and more of the tail of the distribution enters into the abnormal range. For a given change in the mean, there is a larger change in response for $p_0 = 0.05$ than for $p_0 = 0.001$ (because the slope of the bell curve is greater at larger p_0). Stated another way, a smaller change in the mean is necessary to produce a given increased risk (e.g., a BMR of 10%) for a larger p_0. Since the response is being measured relative to untreated animals, a small change in mean corresponds to a lower dose.

Note also the differences in the model-predicted MLEs and BMCs for fixed p_0 and BMR. The differences between the model predictions provide some indication of the model uncertainty or model-dependency of the dose–response modeling results. A part of that model dependency is explained by the fact that the continuous Weibull model allows plateauing, while the power model does not. Generally, the further away from the experimental doses the BMDs are, the greater the model dependence.

Assuming normally distributed variability, the combination of a p_0 of 0.05 and a BMR of 0.1 is equivalent to defining the BMD as the lower bound on dose that results in a change in the mean response equal to 0.6 times the standard deviation (Crump, 1995). Kavlock *et al.* (1995) found that, for a fetal weight endpoint, a BMR defined as $sd_0/2$ yielded BMDs that were on average similar to the corresponding NOAELs for a set of developmental endpoints. Thus, in the absence of additional information (e.g., toxicological consensus on what values of a continuous variable constitute abnormal or adverse observations—that is, on the definition of "biologically significant" changes), the combination of $p_0 = 0.05$ and BMR = 0.1 might be an appropriate choice to use with the hybrid approach for dose–response modeling of continuous endpoints.

Modeling of Oral Studies

Toxicity data appropriate for modeling are available from chronic, subchronic, and reproductive and developmental studies employing oral exposure. Table 5 provides summary information about the studies/endpoints that were considered in this investigation. The chronic and subchronic exposures resulted in decreased body weight, pneumonitis, and/or immunotoxic responses. Prenatal and neonatal mortality increased following *in utero* exposures.

Body weight. Decreased body weight was modeled for a 2-year dietary study in rats with nickel sulfate (Ambrose *et al.*, 1976), and for a gavage study in which rats were dosed for 90 consecutive days with nickel chloride (American Biogenics Corporation, 1988). For both of these studies, several different modeling approaches were considered (see Methods for Modeling and Table 6).

Decreased body weight following subchronic exposure to nickel chloride (American Biogenics Corporation, 1988) could be well represented by the continuous endpoint models (Table 6; Fig. 5). The plateau of the Weibull model appears to be more consistent with the data, but, with only three dose groups, the linear representation provided by the power model cannot be ruled out. Satisfactory fits for both models were also obtained for decreased body weight data following chronic exposure to nickel sulfate (Ambrose *et al.*, 1976) (Table 6).

Interestingly, lower BMDs were obtained in the subchronic American Biogenics Corporation study than for the corresponding model with the chronic Ambrose study. This difference may reflect higher toxicity of nickel chloride compared to nickel sulfate, or higher toxicity via the gavage route compared to dietary administration. Because the same differences were seen for the MLEs as for the BMDs, these differences do not appear to be driven by the uncertainty associated with lower bound calculations or related to dose-selection issues.

Because individual animal data were available for the American Biogenics Corporation (1988) study, the data were also quantalized using the same cut-point as used in the hybrid modeling, and modeled as quantal data. This allowed a direct comparison between modeling based on the actual number of low-body-weight animals, and modeling using the hybrid approach that predicts the probability of low-body-weight animals based on the variability around the mean. There are some differences in the maximum likelihood estimates of the dose corresponding to a 10% increase in incidence that depended on whether the data were quantalized before modeling or treated as continuous observations. Although the same Weibull model was used in both approaches, different MLEs were obtained due to slight differences in the maximization of the likelihood for the two approaches. In the case of the quantalized data, the likelihood that is maximized is the product of the dose-specific probabilities of observing the quantalized results, which is based on binomial probability theory. For the continuous data, the likelihood function involves differences between the observed means and the implicit function for the change in mean as a function of dose [see Eq. (3)] and is based on normal theory. Although asymptotically (for large sample sizes) one might expect these two approaches to converge, for the sample sizes available, there were some apparent differences. The BMD estimates (representing lower bounds on the maximum likelihood estimates) show much closer agreement.

Draft U.S. EPA guidance (USEPA, 1996b) and a peer review of that guidance (USEPA, 1996c) have recommended that biological significance should be the preferred basis in the choice of the BMR. The body weight results provide a particularly relevant basis for starting to carefully consider some of the issues associated with specifying biologically significant changes, where the concept of biological significance relates to the identification of a certain degree of change in a continuous measure that is considered indicative of abnormality or adverse response. At issue are questions concerning whether biological significance should be based on group-level observations (corresponding, in our analyses, to BMDs based on a 10% decrease in the mean body weight), which appears to be the commonly accepted practice, or on changes in incidences of individual adverse responses (corresponding to the hybrid approach).

The difference between these two approaches can be illustrated simply by assuming a symmetrical distribution of body weight and that an effect on body weight is evenly distributed in the population. In this case, a 10% decrease in the mean corresponds to a 10% decrease in body weight for 50% of the animals. A dose that affects 50% of the animals is almost certainly greater than a dose that causes the 10% increased risk that is the basis for the BMR in the hybrid approach. As expected from this analysis, BMDs based on current toxicological practice (i.e, a 10% decrease in the mean response being adverse) resulted in higher MLEs and BMDs than those estimated based on a 10% increase in the incidence of "low-weight" animals (Table 6). (For the purposes of comparison, "low-weight" was defined to be 10% below the control group mean.) A BMD of 1.5–4.9 mg Ni/kg/day was calculated for the American Biogenics Corporation data when the BMR was defined in terms of a 10% increase in incidence of low weights. In contrast, the BMD was 17 mg Ni/kg/day (power model or polynomial model) based on a 10% decrease in the mean.

TABLE 5
Oral Nickel Data Considered for Modeling

Reference: study description	Dose (mg/kg/day)	Effect observed/NOAEL (mg/kg/day)	Modeled	Comments
American Biogenics Corporation (1988) 0, 5, 35, or 100 mg/kg/day nickel as nickel chloride hexahydrate by gavage in water for 92 days. All high-dose animals died. At the mid dose, 6/30 males and 8/30 females died; deaths of 3/6 males and 5/8 females were attributed to gavage errors, based on histopathological analysis.	0, 5, 35, 100	Decreased body weight in males/NOAEL of 5	Yes	Food consumption decreased, but not related to problems with palatability. Effects on organ weight secondary to body weight change. High dose not modeled because all animals died.
		Increase in white blood cells in males at the interim termination/NOAEL of 5	No	Statistically and clinically significant response, but no effect at the final termination
		Pneumonitis in males/unknown	No	No histopathology data for low-dose animals
		Pneumonitis in females/unknown	No	No histopathology data for low-dose animals
Ambrose *et al.* (1976) Male and female rats given 0, 100, 1000, 2500 ppm Ni as nickel sulfate in diet for 2 years.	0, 5, 50, 125, assuming a food factor of 0.05	Decreased body weight in males/NA	No	Decrease consistent with time only at the high dose.
		Decreased body weight in females/ NOAEL of 5	Yes	Study limited by high mortality (>80%) in controls at 104 weeks. Therefore, body weight at 78 weeks was modeled.
Smith *et al.* (1993) Two-generation reproduction toxicity study of rats given 0, 10, 50, or 250 ppm nickel as nickel chloride in drinking water.	0, 1.33, 6.80, and 31.63 (overall average)[a]	Litters with dead pups at birth in first and second breeding/NOAEL of 6.8 for this endpoint[b]	Yes	Data for second breeding modeled due to higher responses at the low and mid doses.
		Total number of dead pups/LOAEL of 1.3	No	Not reported on a per litter basis.
		Percentage dead pups per litter/LOAEL of 1.3	No	No measure of variability was provided.
Research Triangle Institute (1988) Two-generation reproduction study of rats given nickel chloride at 0, 50, 250, or 500 ppm in drinking water	0, 6.0, 25.0, 41.8[c]	Decreased live pups/litter in F1a generation/NOAEL of 41.8	Yes	
		Decreased live pups/litter in F1a generation/Significant effect at all doses	No	Effect at low doses was not chemical-related, since the room temperature was 10°C higher than normal, and this can affect fetal development (IRIS, 1996).
		Neonatal mortality (Postnatal Day 1–4) in the F1a litter/NOAEL of 41.8	Yes	Effect also observed in F2b litter, but the response did not reach statistical significance.
Dieter *et al.* (1988) Female mice given 0, 1000, 5000, or 10,000 ppm nickel sulfate in drinking water for 180 days. Study focused on effects on immune system.	0, 44, 108, 150 (measured dose)	Decreased lymphoproliferative response/LOAEL of 44	Yes	Considered secondary to effects on the myeloid system (decrease in bone marrow cellularity and in granulocyte-macrophage proliferative responses), because other immune parameters were not affected. Modeled because it was the most sensitive effect.

[a] Average doses were about 20% lower during prebreeding and gestation, and about 60% higher during lactation, due to higher water consumption during lactation.

[b] Supported by results for other measures of pup death. The overall study NOAEL may be lower than 6.8 mg/kg/day, based on the other two measures of perinatal mortality, but the identification of a NOAEL is problematic due to the lack of a clear dose–response for dead pups/litter at the low doses.

[c] The average nickel consumption reported by the authors varied by more than a factor of 2, with the highest consumption at the beginning of the premating exposure and during the latter part of the lactation period. As a conservative estimate, the exposure during gestation, which was on the low end of overall exposure levels, was used for modeling. This choice also takes into account the possibility that gestational exposure alone could account for the observed effects.

While a 10% decrease in the mean body weight may be an adequate cutpoint for determining when a group of animals can be considered adversely affected, it may be a poor indicator of a biologically significant change for an individual animal. Although this point is illustrated here using body weight data, similar problems are likely to arise in other attempts to use biological significance as the basis for the choice of BMR, because biological significance in animals is often defined in terms of changes at the group level, while the probability of an effect is modeled for an individual.

Consider also that the current definition of adversity for body weight is consistent only as an indicator of LOAELs

TABLE 6
Decreased Body Weight following Oral Exposure to Nickel

BMR definition	Power			Weibull			Polynomial		
	MLE	BMD	G-O-F *p* value	MLE	BMD	G-O-F *p* value	MLE	BMD	G-O-F *p* value
American Biogenics Corporation (1988) Decreased body weight at 13 weeks in male rats exposed via gavage to nickel chloride									
10% decrease in mean	21	17	0.15	N/D[a]	N/D	N/D	21	17	**0.15**
10% additional risk of 10% decrease, quantal modeling	N/D	N/D	N/D	4.4	1.5	1.0	4.4	1.5	**1.0**
10% additional risk of 10% decrease, continuous modeling, "hybrid approach"	5.9	4.9	0.17	2.4	1.5	N/A[b]	N/D	N/D	**N/D**
Ambrose *et al.* (1976) Decreased body weight at 78 weeks in female rats exposed in diet to nickel sulfate[c]									
10% decrease in mean	**42**	**36**	**0.60**	**N/D**	**N/D**	**N/D**	**42**	**36**	**0.60**
10% additional risk of 10% decrease, continuous modeling, "hybrid approach'	**19**	**15**	**0.68**	**10**	**6.8**	**0.95**	**N/D**	**N/D**	**N/D**

[a] N/D, not done; this form of the model does not exist.
[b] N/A, not available. Goodness-of-fit *p* values could not be calculated for some models due to insufficient degrees of freedom.
[c] Quantal modeling could not be conducted for Ambrose *et al.* (1976), because individual animal data were not available.

(ignoring, for the sake of this discussion, the inconsistencies inherent in the LOAEL/NOAEL approach that were mentioned in the introduction). The 10% decrease in mean weight identifies the point at which adversity (on a group level) has become apparent. If, as above, one contemplates the definition of a biologically significant cut-point that corresponds to a 10% decrease in mean weight, it may be the case that the resulting BMRs result in estimates (MLEs and/or BMDs) that are more like LOAELs than NOAELs.

This point is illustrated by the two body weight data sets modeled for oral exposure to nickel. In the Ambrose *et al.* (1976) study, the NOAEL was 5 mg Ni/kg/day, with a 5% decrease in body weight, and the LOAEL was 50 mg Ni/kg/day, with a 20% decrease in body weight. The BMD based on a 10% decrease in the mean was 36 mg Ni/kg/day. As expected, the BMD was between the LOAEL and the NOAEL, but it was closer to the LOAEL. In the American Biogenics Corporation (1988) study, the percentage decrease in body weight at the NOAEL (5 mg Ni/kg/day) was 5%, while the percentage decrease at the LOAEL (35 mg Ni/kg/day) was 18%. The BMD was estimated to be 17 mg Ni/kg/day. Even though the "conservatism" of using lower bounds in the definition of the BMD has already been incorporated, BMDs based on a 10% decrease in mean weight were higher than NOAELs and closer to LOAELs. A hybrid modeling approach and definition of a biologically significant cut-point that had as its goal the matching of a 10% decrease in mean weight would be expected to maintain a similar relationship to the NOAELs and LOAELs.[4] Conversely, a BMD based on a 10% increase in the incidence of low-weight animals may be overly conservative compared to standard toxicological practice that defines adverse effects on body weight based on changes in the mean.

The most appropriate definition of the BMR for decreased body weight relative to controls, or for most continuous endpoints, has yet to be determined, but it is a crucial issue. Early applications examining developmental toxicity studies (Allen *et al.*, 1994a,b; Kavlock *et al.*, 1995) have defined BMRs to determine where there is a correspondence, on average, between NOAELs and resulting BMDs; it is not clear how those results will generalize to endpoints from other experiments. Little work has been conducted to systematically investigate the correspondence between different BMR definitions and NOAELs for studies on endpoints other than developmental toxicity. Haag-Grönlund *et al.* (1995) compared NOELs and LOELs reported by study authors for systemic toxicity and neurotoxicity of trichloroethylene with different BMDs, and found that 42% of NOELs were higher than the corresponding quantal BMD10 (10% response) values. This correspondence is consistent with the results from the developmental studies.

Other endpoints. The models implemented for the hybrid approach to modeling the continuous lymphoproliferative effect (Dieter *et al.*, 1988) were generally unsuccessful in representing the observed dose–response pattern, as indicated by the low *p*-values associated with goodness of fit for that endpoint (Table 7). The discrepancies between the observed and predicted mean responses occurred mostly at the higher dose levels.

The prenatal and neonatal mortality data were fit well by the models, whether the data were available in quantal form (number of litters with dead pups, Table 8; Fig. 6) or continuous form (percentage mortality per litter, Table 7). For the latter representation, the power model provided a clearly superior fit,

[4] This is because, for a standard study design, if the MLE has as its target a 10% change, the lower bound, the BMD, will still be associated fairly consistently with percentage changes in body weight only slightly lower, maybe 7–8, decreases.

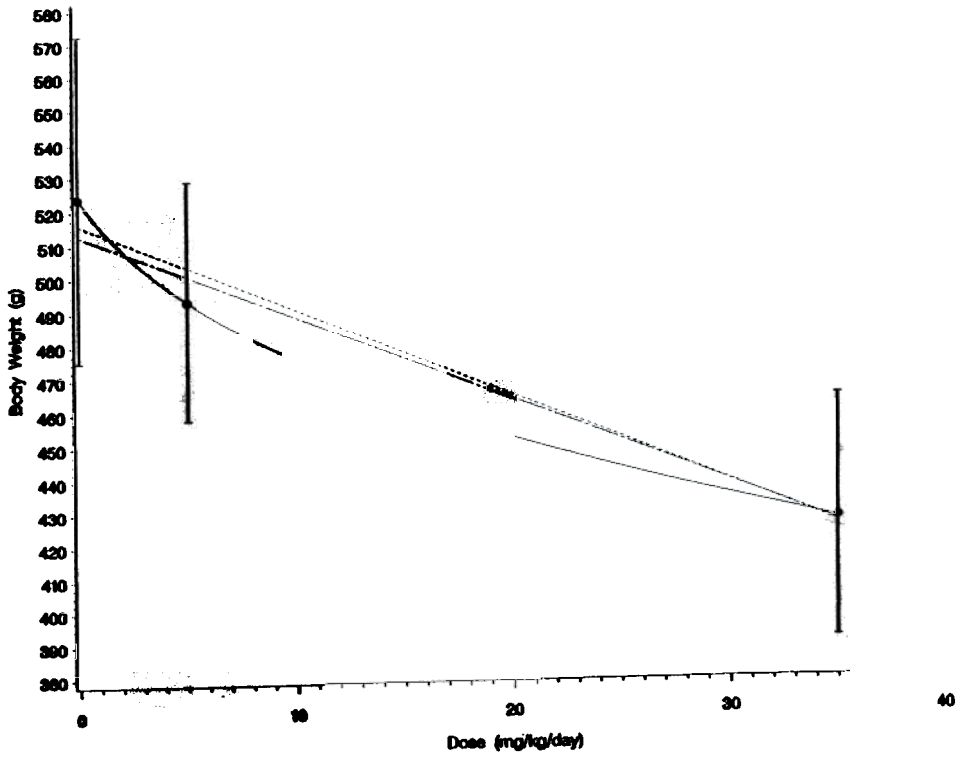

FIG. 5. Graphical display of the results of benchmark modeling of decreased body weight in male rats administered nickel chloride by gavage for 13 weeks (American Biogenics Corporation, 1988). Data points represent the mean ± standard deviation. (—) Weibull model, (- - -) power model, modeling conducted using the hybrid approach with the cutpoint (x_0) fixed. (– – –) modeling conducted with the BMR defined as a 10% decrease in the mean; identical results were obtained for the power and polynomial models.

affecting the MLEs but, interestingly, not the BMDs (Table 7). In this case, the lower bounds appear to be more stable to choice of model. The prenatal mortality endpoint appears to be almost as sensitive an indicator of the toxicity of oral nickel chloride exposure as decreases in body weight—the MLEs and BMDs for the latter are only slightly less than those for the former.

CONCLUSION

This paper illustrates some of the issues and problems that may arise in conducting a noncancer risk assessment that includes benchmark dose modeling. It should be apparent that although dose–response modeling, and therefore benchmark dose estimation, is a statistical methodology, toxicological judgment remains central to the risk assessment procedure, starting with identification of studies and endpoints to model, and continuing through comparison and interpretation of modeling results. Thoughtful application of dose–response modeling provides results that are consistent with toxicological judgments and can strengthen, quantitatively, the conclusions associated with sound scientific thinking. As a specific example, use of the hybrid modeling approach for continuous endpoints

TABLE 7
Continuous Results for Oral Exposure to Nickel

Model	P0	X0	BMR	MLE	BMD	G-O-F p value
			Dieter *et al.* (1988) Lymphoproliferative response in female mice			
Weibull, P0 fixed	5.00×10^{-2}	1.70×10^{1}	1.00×10^{-1}	8.32×10^{0}	4.07×10^{0}	5.20×10^{-2}
K Power, P0 fixed	5.00×10^{-2}	1.48×10^{1}	1.00×10^{-1}	3.93×10^{1}	2.78×10^{1}	8.55×10^{-3}
			RTI (1988) % mortality/litter F1a Postnatal Day 1–Postnatal Day 4			
Weibull, P0 fixed	5.00×10^{-2}	5.70×10^{0}	1.00×10^{-1}	2.58×10^{1}	2.47×10^{1}	2.54×10^{-2}
K Power, P0 fixed	5.00×10^{-2}	5.91×10^{0}	1.00×10^{-1}	3.30×10^{1}	2.66×10^{1}	2.16×10^{-1}

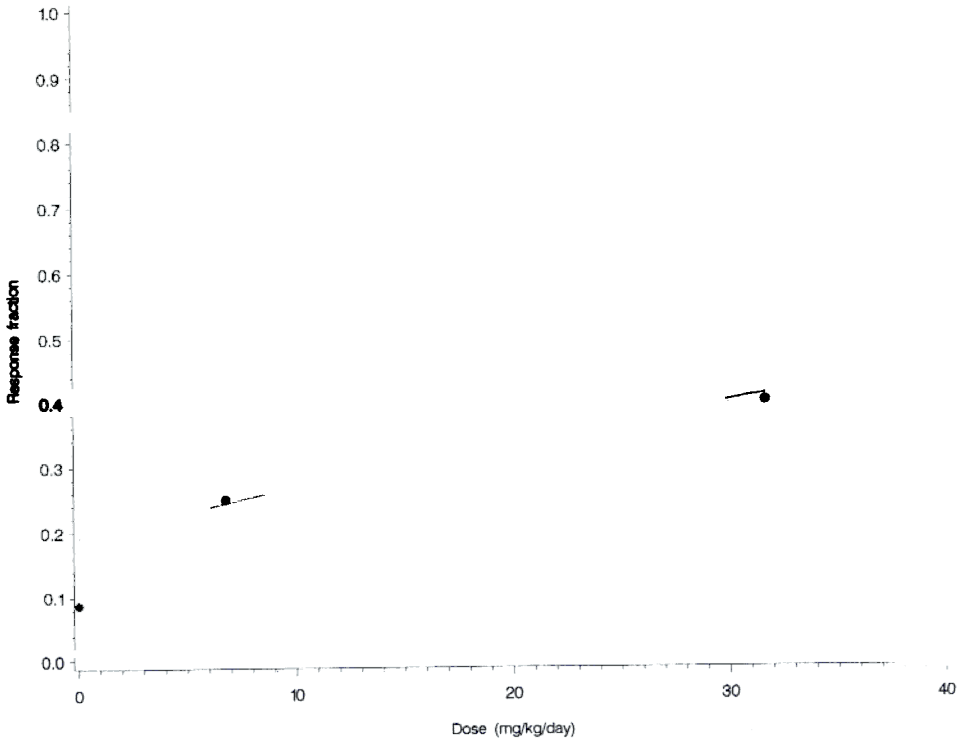

FIG. 6. Graphical display of the results of benchmark modeling of the number of litters with dead pups at birth (Smith *et al.*, 1993). Identical results were obtained for the polynomial and Weibull models.

allows such endpoints to be compared directly to the quantal endpoints, in terms of the risks of adverse responses at particular doses or concentrations.

The data set available for various nickel compounds is extensive and complex. By considering several nickel compounds together, we have been able to consider cross-chemical similarities and differences. Particularly noteworthy were the similar responses and concentration–response patterns that were seen for nickel subsulfide and nickel sulfate in the subacute studies. Even though information on dose–response relationships was sketchy at best, BMCs corresponding to a fixed level of risk were capable of rank ordering the respiratory toxicity of the oxide compared to the subsulfide and sulfate compounds, and were consistent with toxicological judgment.

Difficulties arise when the lowest response rates are at or near 100%. Because such observations provide little or no information about the shape of the dose–response curve, the most appropriate interpretation of those observations is not clear. Such data sets are quite problematic for both BMD and NOAEL/LOAEL methods and best handled by the acquisition of additional toxicity data. However, both approaches could benefit from data from other sources regarding the shape of the dose–response curve.

Also noteworthy were the patterns observed in the chronic inhalation studies. Again, similar endpoints were observed for all three compounds. In this case, however, similar concentration–response patterns were observed for nickel subsulfide and nickel oxide (at or near 100% response at all nonzero exposure levels). The observed nickel sulfate concentration–response pattern, based on testing conducted at lower exposure levels, provided much more information about the shape of the concentration–response relationship, with at least one exposure

TABLE 8
Quantal Endpoint from Oral Exposure to Nickel

Study	BMR definition	Polynomial			Weibull		
		MLE	BMD	G-O-F *p* value	MLE	BMD	G-O-F *p* value
Smith *et al.* (1993) Number of litters with dead pups	10% extra risk	1.05×10^{1}	4.97×10^{0}	$1.76 \times 10^{-}$	1.05×10^{1}	4.97×10^{0}	1.76×10^{-1}

level showing little change from control and another exposure level associated with a moderate response level. It would be fruitful to investigate the possibilities for integrating information on dose–response patterns for closely related compounds, such as those considered here.

Because of the aforementioned difficulties, the identification of a principal study and critical endpoint for the derivation of a nickel RfC is problematic. However, for chronic exposure, nickel sulfate appears to be the most toxic or comparable to the most toxic of the three compounds. Nickel sulfate produced responses only slightly lower than those resulting from nickel subsulfide exposure, at HEC values about half the lowest tested concentration of nickel subsulfide. Nickel oxide concentrations producing comparable responses were much higher. Therefore, a nickel RfC could be derived based on the most sensitive BMC for chronic exposure to nickel sulfate, 1.7×10^{-3} mg Ni/m^3 for lung fibrosis in male rats. (The application of uncertainty factors and ultimate RfC calculation are not addressed here.) The BMC calculated for olfactory epithelial atrophy in female rats following subchronic exposure was a factor of approximately 3 lower (4.8×10^{-4} mg Ni/m^3), but the response incidence for this endpoint was lower in the chronic study, suggesting that recovery may have occurred. An additional reason that nickel sulfate may be more appropriate than nickel subsulfide as the basis for the nickel RfC is that nickel sulfate is a more environmentally relevant compound. A case could be made that nickel oxide is less toxic, and a less conservative RfC may be appropriate for nickel oxide. However, better exposure–response data in the low-concentration region would be needed to support a separate RfC for nickel oxide.

Identification of an appropriate basis for the nickel RfD is not as difficult. Approximately equivalent sensitivity, in terms of the values of the BMDs, was observed for the developmental and systemic endpoints; a BMD of 4.1 mg Ni/kg/day was calculated for decreased lymphoproliferative response in female mice exposed for 180 days (Dieter *et al.*, 1988), and a BMD of 4.97 mg Ni/kg/day was estimated for increased number of litters with dead pups in the first generation of a two-generation reproductive study (Smith *et al.*, 1993). These values are consistent with the BMDs derived for other oral-exposure endpoints, which range from about 1.5 mg Ni/kg/day for some versions of the body weight analyses to 25 mg Ni/kg/day for the (apparently) less sensitive neonatal mortality endpoints (RTI, 1988). The BMDs differ by only about a factor of 2 from their corresponding MLEs and thus there is no suggestion that the values of the BMDs are artifacts of poor data. Because Dieter *et al.* (1988) considered the decreased lymphoproliferative response to be an effect secondary to effects on the myeloid system, the BMD associated with the direct developmental effects (prenatal mortality) might be preferable for regulatory purposes. In any case, a BMD of about 4 or 5 mg Ni/kg/day (a value not inconsistent with the NOAELs reported by various study authors) would be appropriate for regulatory application.

Note, in closing, that this analysis of nickel compounds has been done in the context of several, more or less independent, chemical-specific BMD investigations. Because there are always case-specific considerations affecting the application of the BMD approach, such analyses are worthwhile and informative. Moreover, in the course of such assessments, one identifies issues related to BMD applications that have more general implications. We have addressed some of the issues that were salient to the analysis of nickel (e.g., cross-chemical and cross-endpoint comparisons, choices associated with the definition of the BMD for continuous endpoints, and, more specifically, the considerations appropriate to the incorporation of "biological significance" for that definition). The resolution of these issues can be accelerated by the accumulation of a number of case studies, such as the one presented here, for which analytical options are compared and tested against toxicological judgment.

ACKNOWLEDGMENTS

Stephen Eric Brooks and Cynthia Van Landingham provided expert programming, data management, and graphic support. Thanks go also to Woodrow Setzer, Gary Foureman, and Karen Gan for useful comments.

REFERENCES

Alexeeff, G. V., Lewis, D. C., and Ragle, N. L. (1993). Estimation of potential health effects from acute exposure to hydrogen fluoride using a "benchmark dose" approach. *Risk Anal.* **13**, 63–69.

Allen, B. C., Kavlock, R. J., Kimmel, C. A., and Faustman, E. M. (1994a). Dose response assessments for developmental toxicity: II. Comparison of generic benchmark dose estimates with NOAELs. *Fundam. Appl. Toxicol.* **23**, 487–495.

Allen B. C., Kavlock, R. J., Kimmel, C. A., and Faustman, E. M. (1994b). Dose response assessments for developmental toxicity: III. Statistical models. *Fundam. Appl. Toxicol.* **23**, 496–509.

Allen, B. C., Strong, P. L., Price, C. J., Hubbard, S. A., and Daston, G. P. (1996). Benchmark dose analysis of developmental toxicity in rats exposed to boric acid. *Fundam. Appl. Toxicol.* **32**, 194–204.

Ambrose, A. M., Larson, P. S., Borzelleca, J.F., and Hennigar, G. R., Jr. (1976). Long term toxicologic assessment of nickel in rats and dogs. *J. Food Sci. Technol.* **13**, 181–187.

American Biogenics Corporation. (1988). Ninety day gavage study in albino rats using nickel. Study 410-2520. Final report submitted to USEPA, Office of Solid Waste, by Research Triangle Institute and American Biogenics Corporation under Contract 68-01-7075.

Asgharian, B., Wood, R., and Schlesinger, R. B. (1995). Empirical modeling of particle deposition in the alveolar region of the lungs: A basis for interspecies extrapolation. *Fundam. Appl. Toxicol.* **27**, 232–238.

Barnes, D. G., Daston, G. P., Evans, J. S., Jarabek, A. M., Kavlock, R. J., Kimmel, C. A., Park, C, and Spitzer, H. L. (1995). Benchmark dose workshop: Criteria for use of a benchmark dose to estimate a reference dose. *Regul. Toxicol. Pharmacol.* **21**, 296–306.

Benson, J. M., Burt, D. G., Cheng, Y. S., Eidson, A. F., Gulati, D. K., Hahn, F. F., Hobbs, C. H., and Pickrell, J. A. (1990). Subchronic inhalation toxicity of nickel subsulfide to rats and mice. *Inhal. Toxicol.* **2**, 1–19.

Benson, J. M., Burt, D. G., Carpenter, R. L., Eidson, A. F., Hahn, F. F., Haley, P. J., Hanson, R. L., Hobbs, C. H., Pickrell, J. A., and Dunnick, J. K. (1988). Comparative inhalation toxicity of nickel subsulfate to F344/N rats and B6C3F1 mice exposed for twelve days. *Fundam. Appl. Toxicol.* **10**, 164–178.

Benson, J. M., Carpenter, R. L., Hahn, F. F., Haley, P. J., Hanson, R. L., Hobbs, C. H., Pickrell, J. A., and Dunnick, J. K. (1987). Comparative

inhalation toxicity of nickel subsulfide to F344/N rats and B6C3F1 mice exposed for twelve days. *Fundam. Appl. Toxicol.* **9**, 251–265.

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Fundam. Appl. Toxicol.* **4**, 854–871.

Crump, K. S. (1995). Calculation of benchmark doses from continuous data. *Risk Anal.* **15**, 79–90.

Dieter, M. P., Jameson, C. W., Tucker, A. N., Luster, M. I., French, J. E., Hong, H. L., and Boorman, G. A. (1988). Evaluation of tissue disposition, myelopoietic and immunologic responses in mice after long-term exposure to nickel sulfate in the drinking water. *J. Toxicol. Environ. Health* **24**, 356–372.

Dunnick, J. K., Elwell, M. R., Benson, J. M., Hobbs, C. H., Hahn, F. F., Haley, P. J., Cheng, Y. S., and Eidson, A. F. (1989). Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. *Fundam. Appl. Toxicol.* **12**, 584–594.

Dunnick, J. K., Benson, J. M., Hobbs, C. H., Hahn, F. F., Cheng, Y. S., and Eidson, A. F. (1988). Comparative toxicity of nickel oxide, nickel sulfate hexahydrate, and nickel subsulfide after 12 days of inhalation exposure to F344/N rats and B6C3F1 mice. *Toxicology* **50**, 145–156.

Gaylor, D., and Slikker, W. (1990). Risk assessment for neurotoxic effects. *NeuroToxicology* **11**, 211–218.

Haag-Grönlund, M., Fransson-Steen, R., and Victorin, K. (1995). Application of the benchmark method to risk assessment of trichloroethene. *Regul. Toxicol. Pharmacol.* **21**, 261–269.

IRIS. (1997). Integrated Risk Information System. Cincinnati, OH. USEPA. 1996. U.S. Environmental Protection Agency.

Jarabek, A. M., Menche, M. G., Overton, J. H., Dourson, M. L., and Miller, F. J. (1990). The U.S. Environmental Protection Agency's inhalation RFD methodology: Risk assessment for air toxics. *Toxicol. Ind. Health* **6**, 279–301.

Kavlock, R. J., Allen, B. C., Faustman, E. M., and Kimmel, C. A. (1995). Dose response assessments for developmental toxicity: IV. Benchmark doses for fetal weight changes. *Fundam. Appl. Toxicol.* **26**, 211–222.

Kimmel, C., and Gaylor, D. W. (1988). Issues in qualitative and quantitative risk analysis for developmental toxicity. *Risk Anal.* **8**, 15–20.

Lovelace Inhalation Toxicology Research Institute. (1986a). Initial submission: Two-week repeated dose inhalation study in F344/N rats with nickel oxide (final report). Submitted by Shell Oil Co. to U.S. EPA, Document ID 88-920002001, OTS0536195.

Lovelace Inhalation Toxicology Research Institute. (1986b). Initial submission: Two-week repeated dose inhalation study in B6C3F1 mice with nickel oxide (final report). Submitted by Shell Oil Co. to U.S. EPA, Document ID 88-920002615, OTS0536515.

Malsch, P.A., Proctor, D. M., and Finley, B. L. (1994). Estimation of a chromium inhalation reference concentration using the benchmark dose method: A case study. *Regul. Toxicol. Pharmacol.* **20**, 58–82.

NTP. (1996a). National Toxicology Program. Toxicology and carcinogenesis studies of nickel subsulfide in F344/N rats and B6C3F1 mice (Inhalation studies). Technical Report No. 453. NIH Publication No. 96-3369.

NTP. (1996b). National Toxicology Program. Toxicology and carcinogenesis studies of nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice (Inhalation studies). Technical Report No. 454. NIH Publication No. 96-3370.

NTP. (1996c). National Toxicology Program. Toxicology and carcinogenesis studies of nickel oxide in F344/N rats and B6C3F1 mice (Inhalation studies). Technical Report No. 451. NIH Publication No. 96-3367.

Pease, W., Vandenberg, J., and Hooper, K. (1991). Comparing alternative approaches to establishing regulatory levels for reproductive toxicants: DBCP as a case study. *Environ. Health Perspect.* **91**, 141–55.

Research Triangle Institute. (1988). Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: Fertility and reproductive performance of the P_0 generation. Final study report (II of III). Report to Office of Solid Waste Management, US Environmental Protection Agency by Research Triangle Institute. RTI Project No. 472U-3228-07, under EPA Contract No. 68-01-7075.

Schroeder, H. A., and Mitchener, M. (1971). Toxic effects of trace elements on the reproduction of mice and rats. *Arch. Environ. Health* **23**, 102.

Smith, M. K., George, E. L., Stober, J. A., Feng, H. A., and Kimmel, G. L. (1993). Perinatal toxicity associated with nickel chloride exposure. *Environ. Res.* **61**, 200–211.

USEPA. (1991). U.S. Environmental Protection Agency. Guidelines for developmental toxicity risk assessment; notice. *Fed. Reg.* **56**, 63798–63826.

USEPA. (1994). U.S. Environmental Protection Agency. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F. Office of Health and Environmental Assessment, Washington, DC.

USEPA. (1995). U.S. Environmental Protection Agency. Proposed guidelines for neurotoxicity risk assessment; notice. *Fed. Regist.* **60**, 52032–52056.

USEPA. (1996a). U.S. Environmental Protection Agency. Guidelines for reproductive toxicity risk assessment. EPA/630/R-96-009. September, 1996.

USEPA. (1996b). U.S. Environmental Protection Agency. Benchmark dose technical guidance document. Risk assessment forum. EPA/600/P-96/002A External review draft.

US EPA. (1996c). U.S. Environmental Protection Agency. Report on the Benchmark Dose Peer Consultation Workshop. EPA/630/R-96/011.