

Development of an occupational exposure limit for *n*-propylbromide using benchmark dose methods

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Abstract

This paper presents the development of an occupational exposure level (OEL) for *n*-propylbromide (nPB) using benchmark dose methods. nPB is a non-ozone depleting solvent, proposed under the Significant New Alternatives Policy (SNAP) for use as a precision vapor degreaser. OELs have generally been developed on the basis of a NOAEL or LOAEL and application of uncertainty factors; this paper represents a departure from historic methods. Six recently completed toxicological studies were critically reviewed to identify (1) toxicologically significant endpoints, (2) dose–response information on these endpoints, and (3) uncertainties and limitations associated with the studies. Dose–response data were compiled and entered into the USEPA’s benchmark dose software for calculation of a benchmark dose (BMD) and a benchmark dose low (BMDL). Once values were estimated for all relevant studies, they were then incorporated into a weight-of-evidence approach to develop a single BMD and BMDL representative of nPB. This approach is similar to that recently taken by USEPA to develop their own recommended OEL for nPB. USEPA’s approach is compared and contrasted with ours, particularly in relation to the application of uncertainty factors (UFs) to generate a final OEL. There are no published criteria for application of UFs in developing an OEL. Although USEPA recommends utilizing a UF of 9, based on intraspecies variability and pharmacokinetic differences between rats and humans, to meet the goal of protecting healthy adult in a workplace setting, no uncertainty factor was deemed necessary for nPB in this paper. Therefore, the BMDL was recommended as the OEL.

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1. Introduction

n-Propylbromide (nPB or 1-bromopropane) is used as an industrial solvent and has been proposed as a substitute for ozone-depleting substances (ODSs; Enviro Tech SNAP Petition, SNAP Docket No. A-91-42 VI-D-138). nPB could gain widespread use as a non-ozone depleting solvent. Over 55 toxicological studies have been conducted on nPB, the majority in the last four years. Early information (published before 1998) was used by the U.S. Environmental Protection Agency

(USEPA) in 1999 to recommend preliminary air concentrations ranging from 50 to 100 parts per million (ppm) as protective of workplace exposure (USEPA, 1999). The bulk of information compiled in the last four years has only recently been reviewed. Since this work was completed, a recommended OEL using the standard NOAEL/LOAEL approach has been published (Rozman and Doull, 2002). Subsequently, the USEPA has conducted their own benchmark dose modeling for nPB with the goal of recommending an OEL, using the available toxicological database (USEPA, 2003). However, the Occupational Safety and Health Administration (OSHA) has not recommended a permissible exposure limit (PEL). In 1999, OSHA recommended

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nPB for testing by the National Toxicology Program (OSHA, 1999). In their nomination, OSHA stated “there is a pressing need to pin down the ‘true’ NOAEL—even better, to use standard study designs and statistical methods to establish a ‘benchmark dose.’” In response to this request, this paper identifies a recommended occupational exposure limit (OEL) for nPB using statistical benchmark dose methods. This work predated both the Rozman and Doull (2002) and USEPA (2003) studies.

This paper critically evaluates the toxicological database compiled for nPB, focusing on toxicity in humans. Using USEPA’s benchmark dose software (BMDS) Version 1.3 (USEPA, 2001a), a weight-of-evidence (WOE) approach was applied to the toxicity data to develop a recommended OEL. To fulfill this purpose, the literature on nPB was critically reviewed, and benchmark dose low (BMDL) concentrations were identified for various endpoints from the studies using the USEPA software. From these data, a single endpoint was selected upon which to base the OEL, and the use of an uncertainty factor (UF) was evaluated based on the strength of the toxicological database. Finally, an OEL was developed, and compared with the values developed by USEPA and Rozman and Doull.

1.1. BMDL and the traditional approach to occupational exposure levels

Traditionally, OELs are developed by identifying the lowest dose without deleterious effects (no-observed adverse effect level; NOAEL) and dividing by a series of UFs. In the absence of a NOAEL, a lowest-observed adverse effect level (LOAEL) is used (Fig. 1). This approach depends entirely on the doses used in the critical study. It ignores responses at other dose levels, and does not attempt to interpolate response as a function of dose. Advantages of the BMD approach are discussed later in this paper.

The BMDL resulting from this approach is more accurate than a NOAEL or LOAEL. The BMDL is typically calculated using the upper 95% confidence limit on

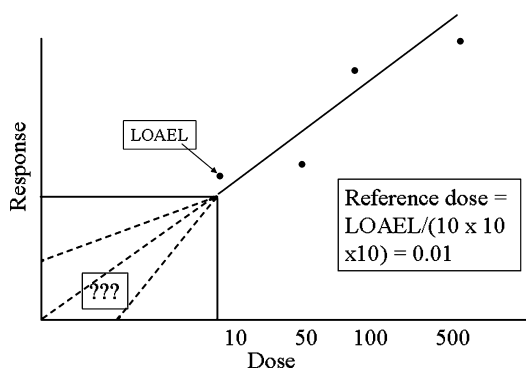


Fig. 1. Traditional approach example using NOAELs/LOAELs.

the dose–response curve (Fig. 2). As a result of using the entire dose–response curve, lower UFs can be applied to adjust the BMDL to an OEL. UFs may be entirely unnecessary, depending on the strength of the database. As stated by Klaassen et al. (1996), “proposed [uncertainty] values... can range from the same factors used for the NOAEL to lower values because of increased confidence in the response level and increased recognition of experimental variability caused by the use of a lower confidence bound on the dose.” Because of these advantages, the BMD approach was used in this study.

The approach presented in this analysis is a hybrid, applying the USEPA approach for developing reference concentrations (RfCs) and application of UFs therein, to OEL development. However, the goal of OEL development is to protect workplace exposure, while the goal of an RfC is to protect environmental exposure. Because these goals are different, USEPA methods are necessarily modified. As stated by USEPA (1994) “due to their derivation methods, attendant assumptions, and intended application, they (OELs) represent risk management values, and this distinction with the RfC as a dose–response estimate must be emphasized.” They further state “OELs are generally time-weighted average concentrations of airborne substances to which a healthy worker can be exposed during defined work periods and under specific work conditions throughout a working lifetime, without material impairment of health.” This is different than RfCs, which are relevant to those of any age and health status and are aimed at protecting the most sensitive members of the population, assuming long-term continuous exposures. Use of a BMD adds to the necessity of adapting USEPA’s methods. Furthermore, as discussed later in this paper, the types of reproductive and developmental data applicable to OEL and RfC development differ (i.e., parent and offspring data).

OSHA and the American Council of Governmental Industrial Hygienists (ACGIH) have not formally recommended criteria for the application of UFs to OEL development. The ACGIH develops threshold limit

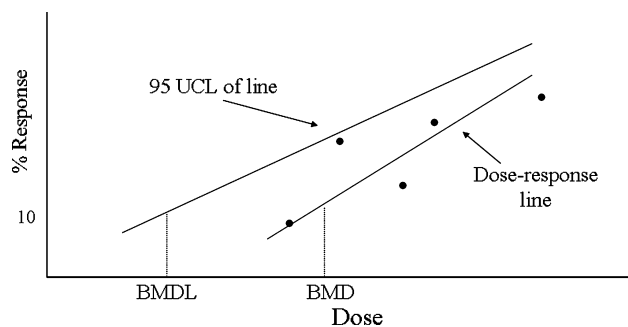


Fig. 2. Example using the benchmark dose approach.

values (TLVs), which have been historically used by OSHA to derive PELs. Based on a review of ACGIH TLVs, the ACGIH generally does not provide the rationale for the use of UFs. Also, OSHA does not typically provide such rationale. Therefore, USEPA criteria for application of UFs are also interpreted for use in this OEL development, as discussed later in this paper.

2. Summary of nPB toxicology

More than 50 toxicological studies have been conducted on nPB. The great majority of these studies were inhalation studies using laboratory rats. A brief overview of nPB toxicity, focusing on the inhalation exposure route, is provided below.

2.1. Toxicokinetics

There is sufficient evidence in both animals and humans that nPB is absorbed across the lung. However, blood–air partition coefficients measured for nPB in both the rat and human indicate a low potential for accumulation of nPB in the body (Clewell et al., 1998). The blood–air partition coefficient for humans is lower than in rats, indicating that relative inhalation absorption is likely higher in rats than humans. There is also sufficient evidence in laboratory animals that nPB is absorbed across the gut following oral administration; however the absorption efficiency through this route has not been measured.

nPB is primarily eliminated unchanged through the lungs in expired air. Once absorbed, nPB partitions predominantly into tissues rich in lipid (e.g., adipose tissue), with substantially lower levels in the liver, brain, and kidneys. nPB is not retained in the body; about 50% of an intraperitoneally absorbed dose is eliminated in expired air within 2 h in rats (Jones and Walsh, 1979). The parent compound and metabolic products are excreted in the urine primarily as mercapturic acids (Clayton and Clayton, 1981; Jones and Walsh, 1979).

2.2. Toxicodynamics

n-Propylbromide is primarily metabolized through conjugation with glutathione, which results in the release of free bromine ions. In vitro nPB studies indicate that oxidation at the C2 and C3 positions occurs before conjugation of the alkyl group with glutathione (Jones and Walsh, 1979; Khan and O'Brien, 1991). Reactive intermediates have not been observed at detectable levels. The metabolic process is primarily through direct conjugation in a Phase II reaction. To a lesser extent, cytochrome P-450 appears to be involved in a Phase II reaction, which is then followed by conjugation with glutathione in Phase II. The same isoenzyme activity

apparently catalyses the reaction in both humans and rats, but human enzyme activity is likely higher (Thier et al., 1999).

Because of the demonstrated presence of an epoxide during the metabolism of 2-bromopropane (and associated carcinogenic activity), the potential for an epoxide intermediate to be involved in nPB metabolism has been conjectured. The replacement of bromine with hydroxyl can occur through epoxidation, which leads to a short-lived reactive intermediate. Based on detected metabolites, it is clear that such a pathway, if present, is so minor that the risk of it significantly contributing to toxicity is negligible (Jones and Walsh, 1979). This is also supported by results of mutagenicity and carcinogenicity in vitro tests (EnviroMed, 1997), summarized later in this section.

Based on existing information and comparative toxicology gleaned from the critical studies reviewed in this report, it is possible that nPB toxicity is linked to saturation of the glutathione conjugation reaction. Micromolar concentrations of nPB have been shown to deplete glutathione (Khan and O'Brien, 1991). A low risk of toxicity may occur at doses below levels needed to saturate the pathway, but once that threshold is reached, toxicity occurs more frequently. Without knowledge of the quantitative reaction kinetics of nPB in vivo, it is difficult to relate a possible threshold dose at the target cells with a known air concentration of nPB. Data from a 90-day study in mice and rats by the National Toxicology Program, to be published in the near future, are expected to elucidate these points.

2.3. Acute inhalation exposure effects

nPB is an acute irritant, causing irritation to exposed eyes, skin, and respiratory and gastrointestinal tracts. Similar to halogenated solvents as a class, nPB has a depressant effect on the central nervous system (i.e., narcosis; Clayton and Clayton, 1981). Exposure may cause headache, dizziness, nausea, vomiting, or drowsiness and loss of consciousness (Enviro Tech, 2000). nPB is weakly narcotic compared to chloroform (Mueller, 1925). The 4-h 50% lethal concentration (LC₅₀) in rats exposed via nose-only inhalation is 6900 ppm, or 34,500 mg/m³ (Elf Atochem, 1997). A whole-body 4-h inhalation LC₅₀ in rats is 14,374 ppm (71,870 mg/m³; Kim et al., 1999).

2.4. Longer-term effects

Several laboratory and clinical studies have been conducted on subchronic nPB exposure of animals and humans. The key animal studies, all based on inhalation exposure, are summarized below. Data from these studies were utilized to develop a BMD.

2.5. Four-week study

Rats were exposed to 1500 ppm (7500 mg/m³) of nPB for 6 h per day, 5 days per week, for 4 weeks via whole-body inhalation (Ohnishi et al., 1999). Results indicated a statistically significant increased frequency of degeneration of the Purkinje cells in the cerebellum, in the fourth week. (Ohnishi et al., 1999).

2.6. Range-finding study

In a 28-day study by ClinTrials BioResearch (1997b), rats were exposed to nPB by whole-body inhalation. Rats were exposed for 6 h daily, 5 days per week, for 4 weeks in four dose groups: 0 (control), 400, 1000, and 1600 ppm. Toxicity was evident in the high-dose group, including mortality (about half of the animals), neurological signs (e.g., tremors, impaired gait, and hind limb weakness), and histopathological changes in the brain (cell vacuolization), spinal cord, and male reproductive system. Other effects on body weight gain, relative kidney weight, blood parameters (e.g., erythrocyte count), and sperm production were seen at multiple dose levels. Brain cell vacuolization may be reversible, and may be an artifact of how the tissues were prepared. Therefore, the toxicological significance of this effect has been questioned (Clewel et al., 1998). This effect has not been reproduced by others (see study summaries below) at similar exposure levels. However, because it is an indicator of central nervous system effects, this endpoint was further evaluated.

2.7. Ninety-day study

In a 90-day study by ClinTrials BioResearch (1997a), rats were exposed to nPB at 0, 100, 200, 400 or 600 ppm for 6 h per day, 5 days per week, for 13 weeks. Similar to the shorter studies previously discussed, this is a weekly exposure of 30 h. However, due to the longer study duration, total exposure was 390 h, or roughly three times the exposure in the 28-day study (ClinTrials BioResearch, 1997b). Fewer effects were noted in this longer-term study, likely due to the lower dose levels used. The only significant effect reported was slight to mild vacuolization in centrilobular liver cells in male animals exposed to 600 ppm, and male and female animals in the 400 ppm dose group. Changes were dose-dependent in the male but not the female. Hepatocyte vacuolization is reversible when chemical exposure ceases. This effect, therefore, is not necessarily adverse, especially at the relatively low levels of severity seen. None of the endpoints observed in the 28-day study were shown to be significant in this longer study, implying that exposure to concentrations in excess of 600 ppm may be necessary before such effects are observed.

2.8. Neurotoxicity study

In this study by Ichihara et al., male rats were exposed to nPB 8 h daily for 12 weeks to concentrations of 0, 200, 400, or 800 ppm via whole-body inhalation (Ichihara et al., 2000a). This is a weekly exposure of 56 h, totalling 672 h of exposure, almost twice the total exposure evaluated in the previously described studies. Also, exposure during this study was 7 days per week, so there was no recovery time for the exposed animals, unlike the previous studies. Therefore, it is not surprising that effects were observed at lower concentrations. The only effect reported at the low dose level (i.e., 200 ppm) was reduced hindlimb grip strength (Ichihara et al., 2000a). At the 400 ppm dose level, forelimb grip strength was also reduced, plasma creatinine phosphokinase (CPK) levels declined, and body weight was suppressed. At the high dose level, motor nerve conduction velocity and distal latency increased, indicating slower nerve conduction. Decreased cerebral weight and axonal swelling of the tibial nerve were also reported at 400 ppm.

2.9. Developmental and reproductive toxicity

Data from the three key studies discussed below were also utilized to develop a BMD for nPB.

2.10. Twelve-week study

In a study evaluating potential reproductive toxicity by Ichihara et al., male rats were exposed to nPB for 8 h daily for 12 weeks to 0, 200, 400, and 800 ppm by whole-body inhalation (Ichihara et al., 2000b). This is a weekly exposure of 56 h, and a total of 672 h of exposure over the study duration. Animals were exposed to almost twice the weekly dose compared to the 90-day study, and a greater maximum dose was administered (ClinTrials BioResearch, 1997a). The only effect seen at the low dose level (200 ppm) was a decrease in seminal vesicle weights. At the 400 ppm dose level, a decrease in number and percentage of motile sperm was reported, which was more pronounced at the 800 ppm dose level. Other effects on spermatogenesis were reported at the 800 ppm dose level. The authors concluded that the primary effect of nPB on the rat was inhibition of spermiation (Ichihara et al., 2000b).

2.11. Range-finding study

Huntingdon Life Sciences (1999) conducted this study to evaluate effects of inhaled nPB on the pregnant and lactating female rat and developing fetus and offspring. Fifty pregnant rats were exposed to 0, 100, 200, 600, and 1000 ppm nPB by whole-body inhalation for 6 h daily from implantation of the zygote through

weaning of F₁ pups. This represents a total of 31 days (or 186h) of exposure for each F₀ animal. The F₁ pups were directly administered nPB on post-weaning days 1 through 7. Therefore, F₁ pups were exposed for 17 days through mother's milk, and an additional 7 days directly, totalling 24 days of exposure.

The only significant finding in the parent generation was an increase in relative liver and kidney weights in the 600 and 1000 ppm groups. In the F₁ generation, male pups in the high-dose group showed a significantly lower post-weaning body weight, and both males and females showed significant decreases in body weight gain post-weaning at the same dose level. In addition, dose-responsive decreases in serum glucose were seen in male pups at all dose levels, and gamma glutamyl transferase (GGT) levels for both male and female pups were statistically significantly increased at 1000 ppm, indicating potential hepatotoxicity. Finally, there was a statistically significant and dose-related increase in male pup relative adrenal gland weights at all doses. No effects on development or reproduction were identified by the authors.

2.12. Two-generation study

This comprehensive study by WIL Research Laboratories evaluated growth, mating, parturition, and lactation of the parental F₀ generation, and the following two generations in rats (F₁ and F₂). Development and survival of both F₁ and F₂ generations were also evaluated (WIL, 2001). The F₀ generation was exposed to nPB via whole-body inhalation 6h daily for at least 70 days prior to mating, and daily throughout the breeding period and post-mating interval. Dose levels were 0, 100, 250, 500, and 750 ppm. This equates to a weekly exposure of 42h, and a total of 420h over this part of the exposure period. [Note that the Ichihara et al. (2000b) reproductive study used an 8-h daily exposure period, and the high dose level (800ppm) was slightly higher than in the two-generation study.]

Beginning on postpartum day 22, F₁ pups were exposed to nPB identically to that used for the F₀ generation (WIL, 2001). In view of central nervous system pathology reported by others, comprehensive evaluation of both F₀ and F₁ animals included histopathological examination of many parts of the brain.

Key findings and comparison with endpoints reported in other studies are itemized below.

- There was complete infertility in the 750 ppm (F₀) group.
- Statistically significant reductions in sperm motility were seen in the 250 (F₁), 500 (F₀ and F₁), and 750 (F₀) ppm group males.
- A significant increase in abnormal sperm morphology was noted in the 500 (F₀ and F₁) and 750 ppm (F₀) group males.

- Decreased numbers of corpora lutea and an increased number of follicular and/or luteinized follicular cysts were observed in the 750 ppm group F₀ females. Interstitial hyperplasia, correlated with interrupted estrous cycles, was also observed in ovaries of the 500 ppm (F₁) and 750 (F₀) ppm group females.
- No histopathological changes in the brain, and no signs of central or peripheral nervous system effects were noted. The authors, therefore, questioned the relevance of changes in absolute brain weight observed in this study (WIL, 2001).
- Minimal to mild centrilobular hepatocyte vacuolization and increased plasma glycogen were noted. These changes were considered reversible and not adverse by the authors.

No significant effects were reported at the 100 ppm dose level in any generation. It should be noted that the exposure patterns in the studies discussed herein vary from typical workplace exposure patterns. For example, the majority of studies had an exposure pattern of 6h daily over the exposure duration. This is a total of 42h of exposure weekly, compared with a typical workplace setting of 8h daily for 5 days each week (a total of 40h of exposure weekly). Overall, however, total weekly exposures are very similar to those in a workplace setting. Therefore, no adjustments to exposure patterns were applied to these studies in OEL development.

2.13. Carcinogenicity

The USEPA, National Toxicology Program, and International Agency for Research on Cancer have not classified nPB with regard to potential human carcinogenicity. Both in vitro studies of human and animal cells and in vivo studies of laboratory animals have been conducted to evaluate potential mutagenicity and carcinogenicity of nPB (Elf Atochem, 1994–1996; EnviroMed, 1997). These findings indicate that in vitro results obtained from human liver cells are consistent with the relative toxicity of other solvents based on whole-animal toxicity testing, but that nPB has less inherent toxicity to humans than 1,1,1-trichloroethane (TCA). In addition, nPB clearly lacks the genotoxic (and hence, carcinogenic) potential of other halogenated solvents such as trichloroethylene (TCE), tetrachloroethylene (PCE), and 2-bromopropane. These findings are consistent with the assumption that any epoxide-forming metabolic intermediate is of negligible significance.

2.14. Occupational/epidemiological studies

A National Institute of Safety and Occupational Health study of 40 industrial workers indicated no

health effects, with concentrations in workplace air of up to 197 ppm (NIOSH, 1999). NIOSH is expected to publish results of other studies on nPB over the next two years.

A recent case report was published on health effects observed in three workers in a single facility using a solvent containing nPB in a spray gun (Ichihara et al., 2002). Based on the data presented, exposure concentrations are unknown but likely in excess of 150 ppm, and durations ranged from as little as two months to one year. The three female workers reported difficulty walking, dizziness, light-headedness, and headache, weakness and numbness in the legs, lower back and genitals, abnormal sensations in the legs, and difficulties with elimination of urine and stool. A staggering gait (ataxia) and decreases in vibration and temperature sensation were observed in the lower extremities of all three women. Physiological tests indicated no impairment to peripheral nerves or any CNS impacts. Based on this evidence, the authors concluded that nPB was likely the causative agent. The evidence is circumstantial at best because (1) no accurate dose levels were reported, (2) other potential causes were not adequately addressed, and (3) inadequate details were provided in the study to form causative conclusions. Moreover, the report did not state whether or not symptoms resolved when nPB exposure declined or stopped, and no follow-up study has been published.

A case report was published in 1999 describing a demyelinating condition predominantly affecting the lower extremities with CNS involvement in one worker exposed to a solvent containing nPB (Sclar, 1999) for up to two months. Based solely on comparison of these findings with the Ichihara et al. (2000a) neurological study in rats, where similar effects were seen at high concentrations (e.g., 800 ppm), the author postulated that nPB was the causative agent. No measures of exposure to nPB (e.g., biomarkers in urine) or other definitive tests were conducted to identify the causative agent, and possible confounding factors were not addressed. In the absence of exposure information, this case study does not provide useful information to address potential causes of the observed toxicity.

3. Benchmark dose methods

Historically, regulatory agencies used NOAELs and LOAELs in combination with UFs as the basis for developing exposure limits and critical toxicity values [e.g., PELs, RfCs, and reference doses (RfDs)]. However, there are many limitations with this approach (Crump, 1984; Gaylor, 1983; Leisenring and Ryan, 1992; USEPA, 1995). Some of the major limitations are listed below (USEPA, 2000):

- The NOAEL/LOAEL is highly dependent on dose selection since the NOAEL/LOAEL is limited to being one of the doses included in the study.
- The NOAEL/LOAEL is highly dependent on sample size, and does not account for the uncertainty in dose–response estimation due to study design.
- NOAELs/LOAELs do not correspond to consistent response levels for comparisons across studies, chemicals, or endpoints.
- The slope (or steepness) of the dose–response curve is not taken into account.
- A LOAEL cannot be used to derive a NOAEL if no NOAEL is defined in a study. Instead, an uncertainty factor of 10 is routinely assigned.
- A NOAEL is not an accurate estimate of a threshold (i.e., no-effect level).

The BMD approach eliminates many of these limitations, and was, therefore, used to develop an OEL for nPB. USEPA developed its BMDS to facilitate the application of BMD methods (USEPA, 2000). Research into model development began in 1995 (USEPA, 2001b). BMDS version 1.3, released in March of 2001, was used to conduct this analysis.

BMD methods are used within USEPA to develop RfDs and RfCs, which are in turn used to set environmental exposure standards for non-cancer human health effects. A stated goal of the BMD method is to “define a starting point of departure (POD) for the computation of a reference value (RfD or RfC) or slope factor that is more independent of study design” (USEPA, 2000). BMD methods involve fitting mathematical models to dose–response data and using results to select a BMD associated with a predetermined benchmark response (BMR). For example, a BMR could be a 10% decrease in relative organ weight, or a 10% increase in the incidence of a lesion or other endpoint. The BMR values used in this nPB analysis are consistent with recent USEPA practice. As stated by USEPA (2000), “the major aim of benchmark dose modeling is to model the dose–response data for adverse effects in the observable range (i.e., across the range of doses for which toxicity studies have reasonable power to detect effects) and then select a “benchmark dose” at the low end of the observable range to use as a ‘point of departure’ for deriving quantitative estimates below the range of observation.”

A 10% response level is conventionally used for dichotomous endpoints (e.g., tumor formation) because it is at the low end of the observable range for many common study designs (USEPA, 2000). For continuous data (e.g., body weight gain), a variety of options can be used to identify a BMR. The convention utilized herein is to select a BMR equal to a change in the mean response equal to one control standard deviation from the control mean. This provides a standardized basis for comparison similar to the 10% response level for

dichotomous data, and generates an excess risk of approximately 10% for the proportion of study individuals either below the second percentile, or above the 98th percentile (depending on the direction of the adverse effect), relative to control individuals (USEPA, 2000). As a result, the BMR for continuous data differs for each endpoint and is a direct function of the variability of responses seen in the control animals.

In general, the BMDS utilizes models that express dose–response information as a function of dose, covariates (if applicable), and a set of value parameters that govern details of the shape of the curve. The models are fitted to a data set by identifying parameter values that adjust the predictions of the model for observed values of dose and covariates to be as close to the observed responses as possible. Such models are considered “non-linear” because the response is not a linear combination of the parameters. They are more difficult than linear models to fit to data, and statistical inference is more approximate than with linear models. Nonlinear models do not necessarily have a biological interpretation. Criteria for final model selection are based almost exclusively on how well the model fits a given data set.

Applying the dose–response models using the BMDS involves four basic steps (USEPA, 2001b): (1) create a data set, (2) select the appropriate model based on the type of data evaluated (e.g., continuous or dichotomous), (3) specify the parameters associated with the model, and (4) run the model and review textual and graphic results. The BMDS provides results for each modeling run that includes a reiteration of the model formula and model run options, goodness-of-fit information, the BMD, and the estimate of the lower 95% confidence limit on the BMD, known as the BMDL. The focus of results discussed below is the BMDL, which is the value used as the POD for the computation of an OEL.

Goodness of fit is represented by a p value calculated by the BMDS. p Values measure the degree to which the observed dose and response data differ from the dose–response predictions. Small p values indicate that the actual data do not fit the model well. USEPA (2000) recommends use of a p value of 0.10 as the critical value for goodness of fit, instead of the typical value of 0.05 used for most statistical tests. They further advise that selecting the best model should include more analysis than solely using the model with the highest p value. For example, USEPA (2000) suggests that, after running the models for a given endpoint, the residuals from the model, data, and model graphs should be examined to ensure that the models adequately describe the data, especially at the low end of the dose–response curve. Then, a “best” model needs to be selected from among those retained. If the BMDL estimates for different models are within a factor of 3 they are considered indistinguishable, and the model with the lowest Akaike

Information Criteria (AIC) value is selected to provide the BMDL. If the BMDLs are not within a factor of 3, USEPA (2000) recommends selecting the model with the lowest BMDL unless it appears to be an outlier. This approach was implemented for each endpoint and study combination identified for selecting a representative BMDL.

3.1. Endpoints considered for BMD analysis

The studies considered for quantitative evaluation included all multi-dose studies previously discussed: the 28- and 90-day studies (ClinTrials BioResearch, 1997a,b), the neurological study (Ichihara et al., 2000a), and the three reproductive/developmental studies (Huntingdon Life Sciences, 1999; Ichihara et al., 2000b; WIL, 2001). For each of these studies, endpoints were selected for BMD modeling on the basis of toxicological significance, relevance to humans, whether effects were test-related, evidence of a dose–response, statistical significance, and severity of effect. Additional guidance on interpretation of reproductive results was provided by USEPA (1996). A total of 60 endpoints across these six studies were evaluated for applicability to BMD modeling. Based on the nature of the significant endpoints, the following categories of endpoints were identified across the studies: (1) reproductive, (2) neurological, and (3) liver and blood.

In general, reproductive and neurological endpoints were the focus of the BMD analysis. Several endpoints in each category were excluded from quantitative analysis using the criteria discussed above. A subset of 38 endpoints from the six studies was evaluated using BMD modeling. These endpoints are shown in Table 1.

After conducting modeling for each of these 38 endpoints, 14 endpoints were eliminated from further consideration because no modeling results for these endpoints met the minimum criteria (e.g., p value for goodness-of-fit of at least 0.10). For each of the remaining 24 endpoints, where at least one model met minimum criteria, the model with the lowest AIC value was chosen to represent that endpoint, consistent with USEPA (2000) recommendations.

4. Results and discussion

BMDs and BMDLs for each of the 24 successfully modeled endpoints were identified and are presented in Table 2. A total of 10 reproductive endpoints across two generations was compiled, along with nine neurological and five liver and blood endpoints. There is a widespread range of values across endpoints, but the majority of values are in the range of 300–400 ppm. The lowest significant BMDL for each group of endpoints ranged from 156 to 263 ppm. Values below

Table 1
Endpoints evaluated using the benchmark dose modeling software

Critical effect	Study reference
<i>Neurological endpoints</i>	
Decreased forelimb grip strength in male rats	Ichihara et al. (2000a)
Decreased hindlimb grip strength in male rats	Ichihara et al. (2000a)
Decreased maximum motor nerve conduction velocity in male rats	Ichihara et al. (2000a)
Increased distal latency in male rats	Ichihara et al. (2000a)
Gray matter vacuolization in male rats	ClinTrials BioResearch (1997b)
Gray matter vacuolization in female rats	ClinTrials BioResearch (1997b)
White matter vacuolization in male rats	ClinTrials BioResearch (1997b)
White matter vacuolization in female rats	ClinTrials BioResearch (1997b)
Decreased plasma creatinine phosphokinase	Ichihara et al. (2000a)
<i>Reproductive/developmental endpoints</i>	
Parent generation (F ₀) endpoints	
Fertility index (both sexes with identical results)	WIL (2001)
Increased number abnormal sperm	WIL (2001)
Reduced sperm motility	WIL (2001)
Decreased sperm count in the epididymis	WIL (2001)
Ovaries—reduced organ weight ^a	WIL (2001)
Decreased sperm count	Ichihara et al. (2000b)
Decreased testosterone level ^a	Ichihara et al. (2000b)
Decreased number of spermatozoa ^a	Ichihara et al. (2000b)
Decreased number of motile sperm	Ichihara et al. (2000b)
Increased percentage of tailless sperm	Ichihara et al. (2000b)
Increased percentage of sperm with abnormal heads	Ichihara et al. (2000b)
Increased number of degenerating spermatocytes per tubule ^a	Ichihara et al. (2000b)
Increased number of degenerating spermatocytes per 100 Sertoli nuclei ^a	Ichihara et al. (2000b)
Increased number of retained spermatids at basal region per tubule ^a	Ichihara et al. (2000b)
First generation (F ₁) endpoints	
Increased number abnormal sperm ^a	WIL (2001)
Increased number unaccounted uterine implantation sites ^a	WIL (2001)
Decreased number viable pups per litter	WIL (2001)
Reduced sperm motility	WIL (2001)
Adrenals—increased relative organ weight ^a	Huntingdon Life Sciences (1999)
Decreased serum glucose levels (males)	Huntingdon Life Sciences (1999)
Increased serum γ -glutamyl transaminase (males) ^a	Huntingdon Life Sciences (1999)
Increased serum γ -glutamyl transaminase (females) ^a	Huntingdon Life Sciences (1999)
Second generation (F ₂) endpoints	
Decreased number viable pups per litter	WIL (2001)
<i>Liver and blood endpoints</i>	
Liver—increased relative organ weight ^a	Huntingdon Life Sciences (1999)
Centrilobular hepatocyte vacuolization	ClinTrials BioResearch (1997a)
Decreased hematocrit level	ClinTrials BioResearch (1997a)
Decreased number of segmented neutrophils	ClinTrials BioResearch (1997a)
Decreased number of monocytes	ClinTrials BioResearch (1997a)
Decreased number of red blood cells ^a	ClinTrials BioResearch (1997a)
Decreased hemoglobin	ClinTrials BioResearch (1997a)

^a No BMDS modeling results met minimum criteria for BMDL development.

156 ppm appear on Table 2, but are not considered significant BMDLs. Results and rationale for selection of significant BMDLs are separately discussed by type of endpoint below.

4.1. Reproductive endpoints

Reproductive endpoints are often the most sensitive endpoints of chemical toxicity, and provide important

information regarding multi-generational effects of a chemical, including teratogenic, fetotoxic, and mutagenic effects. For nPB, reproductive endpoints were analyzed for the parent (F₀) generation in two different studies (Ichihara et al., 2000b; WIL, 2001), and the parent, first, and second generation of the same study (WIL, 2001). The only overlapping significant endpoint in both studies was sperm count in the F₀ rats. The BMDL was 232 ppm in the Ichihara et al. (2000b)

Table 2
Summary of BMD and BMDL estimates for *n*-propylbromide

Endpoint	Study reference	Benchmark dose (BMD; ppm)	Benchmark dose low (BMDL; ppm)
<i>Reproductive</i>			
I. Parent (F ₀) generation			
Sperm count	Ichihara et al. (2000b)	380	232
Retained sperm in seminiferous tubules	Ichihara et al. (2000b)	194	103 ^a
Sperm deformities	WIL (2001)	351	296
Sperm motility	WIL (2001)	343	263
Epididymal sperm count	WIL (2001)	717	585
Fertility index	WIL (2001)	448	356
II. First (F ₁) generation			
Sperm motility	WIL (2001)	261	156
Litter viability	WIL (2001)	280	188
Plasma glucose level (males)	Huntingdon Life Sciences (1999)	554	333
III. Second (F ₂) generation			
Litter viability	WIL (2001)	238	169
<i>Neurological</i>			
Forelimb strength	Ichihara et al. (2000a)	531	345
Hindlimb strength	Ichihara et al. (2000a)	286	214
Motor conduction velocity	Ichihara et al. (2000a)	632	369
Distal latency time	Ichihara et al. (2000a)	416	307
Plasma creatinine phosphokinase (CPK)	Ichihara et al. (2000a)	239	149 ^a
Brain cell vacuolization:	ClinTrials BioResearch (1997a)		
Grey matter (females)		1178	985
White matter (females)		1535	969
Grey matter (males)		922	728
White matter (males)		285	146 ^a
<i>Liver and blood</i>			
Centrilobular vacuolization (males)	ClinTrials BioResearch (1997b)	345	226 ^a
Number of segmented neutrophils (females)	ClinTrials BioResearch (1997a)	1520	1175
Number of monophils (females)	ClinTrials BioResearch (1997a)	1545	1327
Hematocrit (females)	ClinTrials BioResearch (1997a)	642	401
Hemoglobin (females)	ClinTrials BioResearch (1997a)	631	399

Bold values represent recommended BMDL for each type of endpoint. *Abbreviations used:* BMD, benchmark dose; BMDL, benchmark dose low; ppm, parts per million.

^a Not considered a significant BMDL.

study and 585 ppm in the WIL (2001) study (Table 2). The only other significant endpoint successfully modeled in the Ichihara et al. (2000b) study was the number of retained spermatids in the seminiferous tubules of F₀ rats. The BMDL associated with that endpoint was 103 ppm, the lowest of any of the 24 endpoints included in Table 2. This effect was not specifically evaluated by WIL (2001), although several other measures of spermatogonia function were quantified. This endpoint was not statistically significant at the 200 ppm dose level, but was significant at the 400 and 800 ppm dose levels. The resulting BMDL was below the lowest dose tested, and also below a dose level where no effects were reported (i.e., 200 ppm). The BMDL estimated using the retained spermatid endpoint in the Ichihara et al. (2000b) study was not considered appropriate for consideration because it was estimated to be below the NOAEL, and no supporting studies have been conducted to confirm this possible effect.

Therefore, this endpoint was excluded from further evaluation. In their independent BMD analysis, USEPA (2003) also excluded this endpoint from quantitative evaluation.

Reviewing modeling results presented in Table 2, the lowest BMDL obtained for the F₀ generation from the WIL (2001) study was 263 ppm, for a decrease in sperm motility. This was slightly lower than the value based on sperm deformities, and substantially less than values for fertility index and other reproductive endpoints. This BMDL of 263 ppm is recommended as the BMDL representing reproductive endpoints in the parent generation for consideration in developing an OEL. Although the BMDL of 232 ppm for the Ichihara et al. (2000b) sperm count endpoint (Table 2) is lower than this value, the BMDL of 263 ppm is the lowest consistently reproduced value among those evaluated for the parent generation, particularly when compared to an average of the sperm count BMDLs of 232 and 585 ppm.

For the first (or F₁) generation of offspring, three reproductive endpoints were successfully modeled (Table 2). Sperm motility generated the lowest BMDL (156 ppm); the BMDL of 188 ppm, based on litter viability, was slightly higher (WIL, 2001). The BMDL for sperm motility was about 100 ppm lower in the F₁ generation than in the parent generation (i.e., 263 ppm; Table 2). This lowest value of 156 ppm is recommended as the BMDL representing reproductive endpoints in the F₁ generation for consideration in developing an OEL (Table 2).

As shown in Table 2, only one endpoint, litter viability, was successfully modeled for the second (or F₂) generation. The BMDL resulting from this endpoint was 169 ppm, which is consistent with the litter viability BMDL estimated for the F₁ generation (188 ppm). Therefore, this value of 169 ppm is recommended as the BMDL representing reproductive endpoints in the F₂ generation for consideration in developing an OEL, as shown in Table 2.

As previously discussed, the goals of protective occupational and environmental exposure levels are substantially different. Consequently, the type of data evaluated for each application also differ. The following text provides a discussion of these different applications.

The goal of this analysis is to develop a safe exposure level for the workplace. Therefore, the parent generation provides the most relevant values upon which to base an OEL. Additionally, since the presence of pregnant women and nursing mothers in the workplace could lead to fetal exposure, OELs are also intended to protect the fetus and breastfed infant under typical working conditions. However, exposure of rodent F₁ offspring in the two-generation study (WIL, 2001) continued from weaning into adulthood. Given the existing information, it is not possible to separate out impacts to the F₁ generation caused in utero and during suckling versus those caused during independent exposure of pups. This reduces the direct relevance of the results observed in the F₁ generation of this study for OEL development. Such impacts seen in adult F₁ or F₂ generation animals are relevant when discussing environmental exposure. Environmental exposure is expected to occur in all environments over several generations and involve both parents and their offspring. Such exposure is expected to be relatively constant (e.g., up to 24 h daily for residents) compared to adult workplace exposure (i.e., 8 h daily for 5 days per week).

For impacts in adult F₁ or F₂ generation animals such as those documented by WIL (2001) to be directly relevant to a workplace scenario, it would be necessary for both human parents to be exposed to nPB long-term in a workplace environment, conceive while employed there, and continue to work there throughout pregnancy and nursing following delivery. To be consistent with the exposure profile of the two-generation study, their

children would then also need to be exposed from weaning (age 1–2 years) to adulthood and subsequent conception of the F₂ generation. This scenario is highly unlikely to occur in the workplace. By contrast to the environmental setting, results based on adult F₁ generation impacts (under conditions applied by WIL, 2001) and F₂ generations (under any conditions) should, therefore, receive less weight for occupational settings than results from the parent generation. Although adult F₁ and F₂ animal data are clearly applicable to RfC development, they are not directly applicable to OEL development. This is supported by USEPA (1994), “OELs are generally time-weighted average concentrations of airborne substances to which a healthy worker can be exposed during defined work periods and under specific work conditions throughout a working lifetime, without material impairment of health.”

4.2. Neurological endpoints

Neurological endpoints were evaluated in two sub-chronic studies (ClinTrials BioResearch, 1997b, Ichihara et al., 2000a). The BMDLs based on the Ichihara et al. (2000a) study ranged from 149 to 369 ppm, as shown in Table 2. The four significant endpoints from the 28-day study (ClinTrials BioResearch, 1997b) were vacuolization of either gray or white matter in the brain in rats of each sex. Three of the four BMDLs from this study ranged from 725 to 1000 ppm. The fourth BMDL, corresponding to white matter vacuolization in males, was 146 ppm (Table 2). This appears to be an outlier, considering the abundance of evidence suggesting that the sexes are similar in their sensitivity to this chemical. In addition, brain cell vacuolization was not confirmed as an endpoint by the two-generation study (WIL, 2001). Only one male survived in the high-dose group studied by Ichihara et al. (2000a), which further decreases confidence in the BMDL based on brain cell vacuolization in male rats. In fact, as the authors stated in their report on the two-generation study, the toxicological significance of vacuolization in neural tissue is questionable because it is reversible once exposure stops (WIL, 2001). Also as previously noted, this endpoint may be an artifact of how the tissues were fixed. Therefore, the brain cell vacuolization-based BMDLs were not further evaluated. Given the lower BMDLs corresponding to other neurological endpoints (except for the discounted outlying result), this exclusion has no effect on the OEL.

As shown in Table 2, the lowest BMDL of 149 ppm from the Ichihara et al. (2000a) study corresponds to reduced plasma CPK, which is an enzyme used in muscle contraction. The second lowest BMDL derived from this study was 214 ppm, which corresponds to hindlimb strength in rats. Although reduced plasma CPK may be a biomarker for neuromuscular toxicity, it was not

specifically measured in any other critical study. Moreover, the goodness-of-fit p value associated with the model used to develop this BMDL was only 0.104, which marginally exceeds the threshold value of 0.100. This indicates that the model is not robust for the data set associated with this endpoint, and that the BMDL is uncertain. Therefore, reduced CPK was not used to represent nPB neurotoxicity. Instead, the second lowest BMDL of 214 ppm for hindlimb strength is recommended to represent neurological endpoints for consideration in OEL development, as shown in Table 2. The associated goodness of fit p value for this endpoint was 0.60, which is much more robust.

4.3. Liver and blood endpoints

Liver and blood endpoints include measures of blood components (i.e., number of different types of white and red blood cells, and hematocrit and hemoglobin levels) and centrilobular vacuolization of liver cells. The latter was seen in the 90-day study (ClinTrials BioResearch, 1997a) while the blood endpoints were observed in the 28-day study (ClinTrials BioResearch, 1997b). The lowest BMDL for this group of endpoints was 226 ppm for centrilobular vacuolization, the only relevant endpoint from the 90-day study. BMDLs from the 28-day study ranged from 400 to 1325 ppm. The authors stated that centrilobular vacuolization was considered reversible and not adverse (ClinTrials BioResearch, 1997a). Therefore, this is not a toxicologically significant endpoint, and was not further evaluated. Instead, the second lowest BMDL of 399 ppm, for reduced hemoglobin in female rats, is recommended to represent liver and blood endpoints for consideration in developing an OEL, as shown in Table 2.

4.4. Summary of BMDLs

The BMDLs recommended for each of the five groups of endpoints are shown in bold type in Table 2. These values are summarized below:

• Reproductive, parent generation (sperm motility)	263 ppm
• Reproductive, F ₁ generation (sperm motility)	156 ppm
• Reproductive, F ₂ generation (litter viability)	169 ppm
• Neurological (hindlimb strength)	214 ppm
• Liver and blood (hemoglobin)	399 ppm.

These five BMDLs are very similar, and differ by a factor of approximately 2. They incorporate endpoints from three different organ systems, and encompass three generations of test animals. The lowest of these BMDLs is 156 ppm. This value is for sperm motility in the F₁

generation, which was also a significant endpoint in the parent generation. Due to the reproducibility of this endpoint, the rigorous study protocol and thorough documentation (WIL, 2001), and the consistent pattern of toxicity seen with regard to BMDL values, the BMDL of 156 ppm is recommended to represent the toxicity of nPB for purposes of developing an OEL. Because this is based on an endpoint in the F₁ generation, this BMDL should be considered conservative for occupational exposure, in light of the preceding discussion. In their independent BMDL analysis, USEPA (2003) questioned whether data from the F₁ generation were applicable to derivation of a workplace exposure limit, particularly in relation to the potential mechanisms by which nPB exerts its effects on the reproductive system. They stated that the available data do not rule out the possibility that the effects on the F₁ generation occurred as a result of effects on parental germ cells or effects mediated by changes to the endocrine system. However, they concluded that, in view of the lack of mechanistic data on potential *trans*-generational effects of nPB, that use of data from the F₁ generation was appropriate and protective.

These results are remarkably similar to those developed by USEPA in their independent analysis of the nPB database. They used decreased sperm motility in F₀ and F₁ males from the WIL (2001) study, and liver cell vacuolization in male and female rats from the ClinTrials BioResearch (1997a) 90-day study. Their resulting recommended BMDL was 169 ppm, based on the same sperm motility endpoint we used to generate our recommended BMDL. This equated to 177 ppm after dosimetry adjustments. Therefore, in essence the only difference between the OEL recommended in this paper and that recommended by USEPA is in the use of uncertainty factors. This is further discussed below.

4.5. Uncertainty factors

As discussed in Section 1, neither the ACGIH nor OSHA has published guidance on the application of UFs in setting a workplace exposure level. The USEPA offers some guidance for the use of UFs, which are used to downwardly adjust a NOAEL or LOAEL to a RfC or RfD. However, this is applicable to environmental, not workplace exposure. The following discussion provides an adaptation of USEPA's approach. USEPA (1994) clearly states that there are differences in development of RfCs and OELs. They state "the evaluation of toxicity data by agencies deriving OELs may differ from that of EPA with respect to weight-of-evidence classification, application of UFs, and other issues. . . the use of OELs is established to protect the average healthy worker (ages 18–65 years) against the adverse effects of inhaled pollutants to which they are exposed only a fraction of a

day (i.e., during a typical 8-h work shift). Inhalation reference concentrations, however, are relevant to those of any age and health status and are aimed at protecting the most sensitive members of the population, assuming long-term continuous exposures.” While a healthy worker population will have individual variation, such variability is incorporated into the BMD approach by accounting for individual variability in response across the tested animals. The assumption that must be met in order for an uncertainty factor to be used to adjust a BMDL based on individual variability in a healthy population is that the individual variability in that population is wider than that in test animals. There is no inherent rationale to make this assumption. Nevertheless, USEPA used an uncertainty factor of 3 to account for sensitive individuals in their OEL derivation.

Historically in developing RfCs and RfDs, the USEPA uses separate UFs, each of which may be up to 10, to account for each of the following criteria when using the NOAEL/LOAEL method: (1) protection of sensitive individuals within the receptor population, (2) extrapolation of toxicity data from animals to humans, (3) extrapolation of subchronic toxicity data to chronic exposure durations, and (4) extrapolation from a lowest-observed adverse effect level (LOAEL) to a no-observed adverse effect level (NOAEL) to assess toxicity. A modifying factor (MF), generally no higher than 3, is typically also used to account for other considerations such as perceived adequacy of the scientific data. The UFs and the MFs for a given chemical are then multiplied together to provide a total UF, which is then used to derive a chronic RfD. The higher the total UF, the greater the degree of uncertainty and conservatism in the resultant RfD. Given the statement from USEPA that application of UFs differs between RfCs and OELs, each criterion is discussed below for its applicability to an OEL for nPB.

4.6. Extrapolation From LOAEL to NOAEL

Use of the BMD approach negates the need for an UF-related to LOAEL to NOAEL extrapolation (Barnes et al., 1995). Since USEPA also used the BMD approach, they did not apply an uncertainty factor for this extrapolation.

4.7. Protection of sensitive individuals

Workplace exposure typically does not involve sensitive individuals. ACGIH TLVs and OSHA PELs are based on exposure of healthy adult workers, and rarely employ a UF protecting sensitive individuals. Neither agency typically provides information regarding the use of UFs in establishing OELs. Use of an OEL based on F₁ generation data (156 ppm) is adequate to address potential fetal exposure through pregnant workers, par-

ticularly when different human child and F₁ pup exposures are considered.

Data for nPB are available for both animals and humans. In vitro bioassays are available for both human and animal cell cultures (EnviroMed, 1997; Elf Atochem, 1994, 1995). For humans, liver hepatocytes were used for the bioassays, while mouse lymphoma and bone marrow cells were used in separate animal bioassays (without metabolic activation). Comparison of these bioassays indicates that effects are seen at similar levels. These results are supported by the inhalation toxicity studies of rats and mice. In addition, the BMD approach incorporates individual variability because it considers all data across individuals for a given endpoint and study, and develops a dose–response curve encompassing individual variability of responses. Taken together, this weight-of-evidence implies that a UF is neither necessary nor warranted for nPB for protection of sensitive individuals. As previously stated, USEPA used an uncertainty factor of 3 for this extrapolation.

4.8. Extrapolation of toxicity data from animals to humans

Historically, the USEPA has used an animal-to-human UF of 3 for inhalation-based values when deriving RfCs (Barnes et al., 1995). This UF has typically been employed to account for differences across species, and always assumes that a chemical can be more toxic in humans than laboratory animals. In vitro bioassays of nPB have been conducted in both human and animal cells, demonstrating that effects are seen at similar levels. Moreover, in vitro data on human cells correlate well with the BMDLs developed from animal studies. For example, nPB was toxic in human in vitro bioassays at only the highest dose tested (500 ppm in solution, which is essentially the concentration at the target cells in an in vitro study. To reach a 500 ppm level at a target cell in vivo, a much higher air concentration would be required because much less than 100% of an air concentration will reach a target cell; EnviroMed, 1997). The range of significant BMDLs derived from animal data was 156–1328 ppm in air, with a mean BMDL across all 24 endpoints of 448 ppm. This implies that human and animal responses first occur at similar concentrations, which is also supported by the available information on occupational exposure by humans (Ichihara et al., 2002; NIOSH, 1999). No effects have been reported in NIOSH studies on volunteers exposed to nPB at concentrations up to 170 ppm (NIOSH, 1999). No exposure levels were measured by Ichihara et al. (2002) during the time the workers reported symptoms. Air measurements after the affected women left the plant, and ventilation of fume hoods was repaired, indicated that concentrations ranged from 60 to 261 ppm. The authors stated that the actual exposure concentrations during the

period when the affected workers were engaged at the plant were likely much higher, since no ventilation was operating in the building.

USEPA used an uncertainty factor of 3 for this extrapolation, based on their conclusion that there are not data to compare the pharmacodynamics between rodents and humans, despite the information presented above.

An OEL developed for workplace exposure precludes children from being regularly exposed. Although fetuses could be exposed, data from the two-generation study (WIL, 2001) indicate that the developing fetus is not substantially more sensitive to nPB than the mother. Use of a UF of 3 adds unnecessary conservatism to the overall OEL estimate, especially considering the proposed OEL is based on an F₁ generation endpoint not expected to be encountered in actual practice. The BMDL of 156 ppm essentially incorporates a UF of 2 compared with the BMDL for the same endpoint in the more relevant F₀ generation (263 ppm/2 = 131.5 ppm).

An alternate approach would base the OEL on the lowest BMDL of the F₀ generation. Since existing data indicate that human and animal responses to nPB occur at similar concentrations, if a UF were to be used, it should be lower than 3, and no more than 2. Applying this factor to the lowest relevant F₀ generation endpoint, 214 ppm for hindlimb strength in the Ichihara et al. (2000a) neurological study, results in an OEL of 107 ppm.

4.9. Extrapolation from subchronic to chronic exposure duration

For nPB, there are several long-term studies as well as shorter-term subchronic and acute studies. Because long-term studies have been conducted, and the BMD is primarily based on chronic studies, there is no need to incorporate a UF for this extrapolation. Further, comparing the subchronic- and chronic-based BMDLs indicates that use of such a UF is not warranted for this chemical. For example, the range of BMDLs developed from the 28-day study (ClinTrials BioResearch, 1997b), 146–1328 ppm, is similar to those developed from the two-generation reproduction study (WIL, 2001), which range from 156 to 785 ppm. This implies that duration of exposure is not a key factor in nPB toxicity. Instead, the data indicate that the peak air concentration and frequency of exposure (which governs the body's ability to recover between exposures) are the most important factors in determining if toxicity may result.

The recommended BMDL for use in developing an OEL is based on the decline in sperm motility in male rats in the first generation of the two-generation study (WIL, 2001). The BMDL associated with this endpoint is 156 ppm. For the reasons discussed above, a UF is not warranted, as the BMDL of 156 ppm reflects impacts on

the F₁ generation and essentially incorporates an uncertainty factor of 2 from the same endpoint in the F₀ generation. Actual human workplace exposures are unlikely to replicate those of the F₁ generation, where both parents would be exposed to nPB at work full-time, and their children would be exposed daily until adulthood. Therefore, an OEL of 156 ppm is sufficiently protective of worker health. USEPA (2003) did not incorporate an uncertainty factor for this extrapolation.

Using the traditional NOAEL/LOAEL approach and evaluating the same studies discussed herein, a NOAEL of 170 ppm corresponding to headache in humans has been identified (considered a precursor to neurotoxicity; Rozman and Doull, 2002; NIOSH, 1999). This is very close to the recommended BMDL of 156 ppm in this paper. A UF of no greater than 2 was recommended to account for the slope of the dose–response curve, leading to a recommended OEL of 90 ppm based on headaches in humans (Rozman and Doull, 2002). Even with the lack of individual variability incorporated into a NOAEL, these authors concluded that a factor of 2 would “protect nearly all workers” based on the following assumptions:

- Neurotoxicity is the most sensitive endpoint of exposure.
- Headache is a low-dose precursor effect for other neurotoxicity effects in humans (Rozman and Doull, 2002).
- The steepness of the dose–response curve for anesthesia indicates that a factor of 2 will encompass virtually every individual in any realistic population size.
- Rats have similar or higher sensitivity to nPB than humans.

If this approach were used with the lowest F₀ BMDL for neurotoxicity (i.e., 214 ppm; Ichihara et al., 2000a), a UF of two would result in an OEL of 107 ppm, close to the value recommended using the NOAEL/LOAEL approach (Rozman and Doull, 2002). In either event, an OEL of no less than 90 ppm is supported by both approaches.

Overall, USEPA (2003) used an uncertainty factor of 9 to lower their BMDL of 169 ppm to a recommended OEL of 18 ppm (which they suggested could be adjusted to 25 ppm). This is the only difference between the 25 ppm recommended by USEPA and the 156 ppm recommended in this paper. At most, an uncertainty factor of no more than 2 is relevant for this chemical, setting the lower bound of an OEL at 78 ppm, or three times that recommended by USEPA.

5. Conclusion

A relatively large toxicological data base is available for nPB. For purposes of developing an OEL for nPB,

over 60 endpoints were considered across six studies and spanning three generations of test animals. A total of 38 of these endpoints were identified for BMD modeling, and BMDL estimates corresponding to various dose–response models were compiled for 24 of these separate endpoints. Reproductive, neurological, blood, and liver endpoints were quantitatively evaluated. A high variability in the significant BMDLs across endpoints was observed. However, the low end of the range of significant BMDLs associated with each group of endpoints was similar across endpoints. These values ranged from 156 to 263 ppm. Consistent with USEPA, the lowest BMDL of this group, 156 ppm, was used to develop an OEL. This BMDL was derived from data on decreased sperm motility in F₁ generation rats provided in the two-generation reproductive study (WIL, 2001). This endpoint is relevant to humans, represents a sensitive endpoint (i.e., reproduction), is supported by results of animal and human in vitro bioassay results, and is protective based on available occupational data. If the OEL were based on the most sensitive F₀ endpoint observed for nPB, the resulting BMDL would be 214 ppm, for neurological effects in rats (Ichihara et al., 2000a).

Methods recommended by USEPA for application of UFs were modified for OEL development, as opposed to environmental exposure levels. Due to the large toxicology data base, which encompasses varying stages of the life cycle (e.g., neonatal and reproductive), varying exposure periods (from acute to chronic), and studies on several rat strains and some human data, an uncertainty factor is not necessary to protect adult workers. Conversely, USEPA used an uncertainty factor of 9 to derive their recommended OEL of 25 ppm. Uncertainty factors are used to address lack of knowledge regarding the relative sensitivity of a chemical in humans versus animals; there is information on nPB that negates this uncertainty. Acceptable OELs based on this and other studies evaluating the entire nPB data base range from 90 to 214 ppm, incorporating UFs of 1 or 2. The recommended OEL for nPB from this study is 156 ppm, which is near the midpoint of this range.

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