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Quantitative Risk Analysis for N-Methyl Pyrrolidone Using Physiologically Based Pharmacokinetic and Benchmark Dose Modeling

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Establishing an occupational exposure limit (OEL) for N-methyl pyrrolidone (NMP) is important due to its widespread use as a solvent. Based on studies in rodents, the most sensitive toxic end point is a decrease in fetal/pup body weights observed after oral, dermal, and inhalation exposures of dams to NMP. Evidence indicates that the parent compound is the causative agent. To reduce the uncertainty in rat to human extrapolations, physiologically based pharmacokinetic (PBPK) models were developed to describe the pharmacokinetics of NMP in both species. Since *in utero* exposures are of concern, the models considered major physiological changes occurring in the dam or mother over the course of gestation. The rat PBPK model was used to determine the relationship between NMP concentrations in maternal blood and decrements in fetal/pup body weights following exposures to NMP vapor. Body weight decrements seen after vapor exposures occurred at lower NMP blood levels than those observed after oral and dermal exposures. Benchmark dose modeling was used to better define a point of departure (POD) for fetal/pup body weight changes based on dose-response information from two inhalation studies in rats. The POD and human PBPK model were then used to estimate the human equivalent concentrations (HECs) that could be used to derive an OEL value for NMP. The geometric mean of the PODs derived from the rat studies was estimated to be 350 mg h/l (expressed in terms of internal dose), a value which corresponds to an HEC of 480 ppm (occupational exposure of 8 h/day, 5 days/week). The HEC is much higher than recently developed internationally recognized OELs for NMP of 10–20 ppm, suggesting that these OELs adequately protect workers exposed to NMP vapor.

Key Words: **PBPK; benchmark dose; exposure assessment; risk assessment; human equivalent concentration.**

N-methyl pyrrolidone (NMP) is widely used as a solvent or intermediate in the petrochemical processing industry and by producers of electronics, cleaners, and coatings. It also is used in pharmaceutical, agricultural, and photographic chemical applications. It is chemically stable, has a low vapor pressure, is miscible in water, and is often used as a substitute for more toxic chlorinated solvents.

The toxicological database on NMP is well characterized and was recently reviewed by the member countries of the Organization for Economic Cooperation and Development at its 24th Screening Information Data Set Initial Assessment Meeting held in Paris, France, in 2007. This international regulatory community judged NMP a low priority for further work. Briefly, NMP exhibits low acute toxicity with oral and dermal LD_{50} values in rodents of 3600–7700 mg/kg. Oral, 90-day exposures of rats and mice to NMP result in decrements in body weight and food consumption, elevated liver and kidney organ weights, and adaptive liver hypertrophy; the no-observed effect level (NOEL) ranges were 169–217 mg/kg/day (rats) and 229–324 mg/kg/day (mice). NMP is not genotoxic in *in vitro* and *in vivo* bacterial and mammalian test systems. Carcinogenic effects were not observed in rats after long-term exposures to NMP via inhalation or the diet. Liver tumors were observed in mice but only at very high oral doses (> 1000 mg/kg/day).

In developmental and reproductive toxicity studies in rats, reduced fetal/pup body weights have been identified as the most sensitive end points (OECD, 2007). This effect is attributed to the parent compound (Flick *et al.*, 2009; Saillenfait *et al.*, 2007). In developmental studies, the no-observed effect concentration (NOEC) for decrements in fetal body weight associated with inhalation of NMP is 60 ppm (Saillenfait *et al.*, 2003), while the NOELs for oral (gavage) and dermal exposures are 125 (Saillenfait *et al.*, 2002) and 237 mg/kg/day (Becci *et al.*, 1982), respectively. In reproduction studies, the NOEC for a decrement in pup body weight associated with inhalation of NMP is 50 ppm (Solomon *et al.*, 1995; Staples, 1990), while the NOEL for oral (diet) exposures is 160 mg/kg/day (Hellwig and Hildebrand, 1999; Thornton, 1999).

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

Because of its widespread use and potential exposure to worker populations, establishing an occupational exposure limit (OEL) for NMP is important. Currently, multiple countries and agencies have derived OELs for NMP, with values range from 1 (Japan) to 100 ppm (South Africa). The common, default approach to derive an OEL is to adjust an animal study NOEC for an 8-h workday and to divide the duration-adjusted NOEC by uncertainty factors (UFs) to account for deficiencies in the toxicological database and for interspecies and intraspecies extrapolations. These determinations are often made using agency-specific methodologies and assumptions with minimal supporting detail or rationale. OELs can be inappropriate if not updated to reflect recent toxicological studies. The most recent NMP OEL (10 ppm) was published by the Scientific Committee on Occupational Exposure Limits (SCOELs) of the European Commission (SCOEL, 2007). This OEL is consistent with the Workplace Environmental Exposure Level value (10 ppm) published by the American Industrial Hygiene Association (1997) and the Maximale Arbeitsplatzkonzentrationen (MAK) value (20 ppm) established by the Deutsche Forschungsgemeinschaft (2006, German Research Foundation). All three OELs are based on or considered developmental effects (decrements in fetal/pup body weight) in rats.

The usage of default UF methodology is a conservative approach that can yield exposure limits below levels necessary or practical to protect human health. However, uncertainties associated with this default approach can be reduced by a quantitative consideration of interspecies pharmacokinetic differences. PBPK models, which use pharmacokinetic and biologic data to perform tissue-specific dosimetric extrapolations across doses, routes, and species, have been widely used for this purpose (Clewell and Andersen, 1996; Sweeney *et al.*, 2001). PBPK models are used to determine the human equivalent concentration (HEC), which is the external concentration that results in the same internal dose metric noted in animal studies at an effect level, such as the NOEL or the benchmark dose (BMD).

Additionally, the assessment may be further refined by applying BMD methods to characterize the dose-response relationship for chemically induced toxicity. The BMD approach is a preferable alternative to the NOEL/lowest-observed effect level (LOEL) approach, which relies upon a single-dose group and is constrained to exposure concentrations tested in the key study. In contrast, the BMD approach utilizes all the dose-response information from the key study (and can be used to model multiple studies simultaneously) and is not constrained to the tested doses. NOEL and LOEL values are imprecise (i.e., there are an infinite number of NOEL values below an observed threshold and an infinite number of LOEL values above an observed concentration), whereas the BMD approach defines a very specific point in the dose-response curve called the point of departure (POD). The number of animals tested is automatically considered in establishing confidence limits for BMD values, with tighter confidence limits generally defined for larger studies, a key factor that is not generally considered in the NOEL/LOEL approach. In addition, high background response (or in this case a shallow dose-response) will tend to result in higher, less protective, POD using the NOEL/LOEL approach.

The objective of this current study was to construct a validated NMP PBPK model that describes the pharmacokinetics of NMP in pregnant rats and humans, and to use that model, along with BMD analysis, to quantitatively determine an HEC based on NMP-induced decrements in the fetal/pup body weights observed in rat studies.

MATERIALS AND METHODS

PBPK Model Structure

The model structure was developed based on toxicity and pharmacokinetic data. A diagram of the PBPK model structure developed for NMP and its major metabolite, 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), is illustrated in Figure 1. A submodel for 5-HNMP was included because 5-HNMP represents

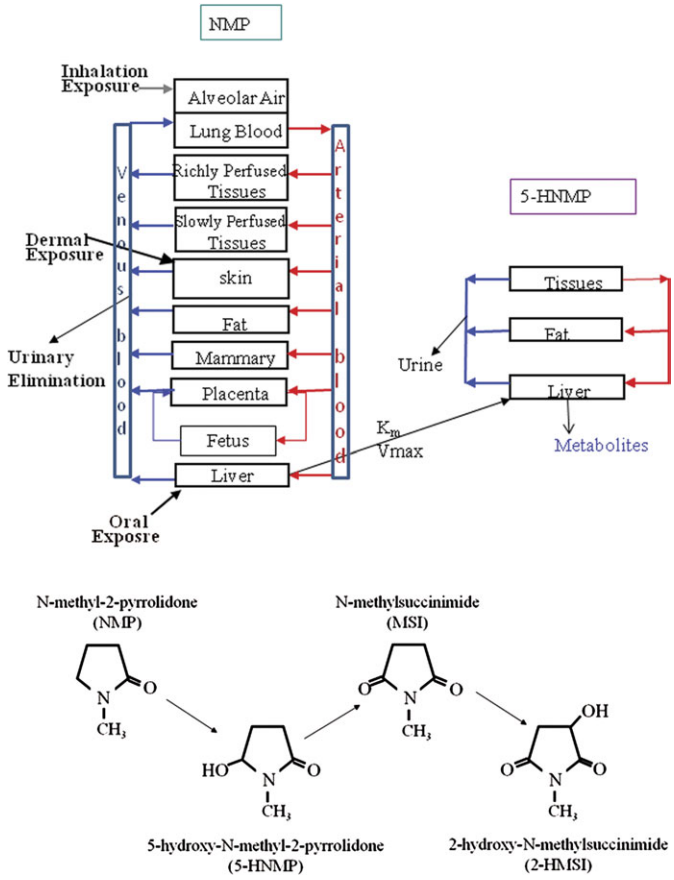

FIG. 1. PBPK model used to describe the disposition of NMP and its major metabolite 5-HNMP in rats and humans following oral, dermal, or inhalation exposures. The lines represent blood flow between compartments (QC, cardiac output [l/h]); Q_i, blood flow to ''*i*'' tissue (l/h). The NMP parent model consists of seven parental tissue compartments, along with arterial and venous blood. The metabolic scheme for NMP (Wells and Digenis, 1988) is also shown.

45–95% of the urinary excretion of NMP-derived radioactivity (Ghantous, 1995; Jonsson and Akesson, 1997; Wells *et al*., 1992), so 5-HNMP may be used as a biomonitor for NMP exposures. The 5-HNMP submodel was simplified by reducing the number of compartments to three: the liver, fat, and combining the rest of the body into a single compartment. In the submodel, the liver was included as the site of metabolism while fat was included because the partitioning differed from the other tissues (see the "Partition Coefficients" section below). Urinary excretion of both NMP and 5-HNMP were considered to occur from the arterial blood via a first-order (rats) or saturable process (humans).

Since a potential end point of concern was the low fetal and pup birth weights in rats, the model includes mathematical descriptions of the important physiological changes occurring in the dam and fetus. The fetal and maternal tissues were described as growing compartments during gestation (Gentry *et al*., 2002, 2003). The pregnancy model was patterned after the isopropanol model developed by Gentry *et al*. (2002), using physiological equations and scaling from that model. Physiological parameter changes during pregnancy were based on literature reports (Hytten and Eg, 1971; ICRP, 1975; Thoresen and Wesche, 1988). Due to the extensive differences between rat and human gestation periods, individual rat and human models were developed, and initial pregnancy model-specific parameters are shown in Table 1. The models were coded using acslXtreme (version 2.5; AEgis Technologies, Huntsville, AL). Cardiac output and ventilation rates were rescaled using the body weight at any given gestational age. Maternal and fetal body weights, placental, mammary and uterine blood flows, and tissue volumes were all increased using time-dependent equations based on elapsed time from the beginning of gestation (Fig. 2). The mammary and uterine tissue compartments were described with flow-limited kinetics. The placental compartment was described as having flow-limited kinetics between the tissue and blood supply but had diffusion-limited kinetics between the tissue and the fetal plasma compartment. The tissue/blood partition coefficient for the placenta was assumed to be similar to that for rapidly perfused tissues, and the diffusion limitation was controlled by a first-order rate constant scaled by total fetal weight to the 3/4 power. All these tissue volumes and blood flows were scaled using the equations presented by Gentry *et al*. (2002).

Physiological and Biochemical Constants

Non-pregnant organ volumes, cardiac output, and tissue blood flows were obtained from the literature (Table 2; Brown *et al*., 1997; Gentry *et al*., 2002). The metabolism of NMP was described using saturable (Michaelis-Menten) kinetics to produce 5-HNMP (Akesson and Jonsson, 1997; Wells *et al*., 1992). The metabolism of NMP has been measured *in vivo* and *in vitro* in the rat (Payan *et al*., 2002) and in human microsomes (Ligocka *et al*., 2003). These rate constants were converted to units utilized by the model (mg/h/kg$^{0.75}$) and used as initial parameters. In order to extrapolate from *in vitro* data to an *in vivo* metabolism rate, protein yields and original liver and body weights were needed but not available, so historical values from our laboratory were used. The maximal rate of metabolism (V_{max}) used for rats was 50% of the estimates from the *in vitro* rates from Payan *et al*. (2002) and approximately two times the estimated rates extrapolated from Ligocka *et al*. (2003) for humans.

The first metabolic step results in the production of 5-HNMP (Akesson and Jonsson, 1997; Wells *et al*., 1992). For the purposes of the PBPK model, additional metabolism of 5-HNMP was also assumed to follow Michaelis-Menten kinetics, which removes 5-HNMP from the model. In addition, 5-HNMP is excreted into the urine. The metabolism of NMP and 5-HNMP was limited to the liver compartment; thus, the 5-HNMP model was linked to the NMP model via the liver.

TABLE 1
Pregnancy-Specific Parameters Used in the PBPK Model for Rats and Humans (initial conditions)

	Rat	Human	Reference
Body weight (kg)	Variable	Variable	Individual article/ ICRP (1975)
Tissue volume (% body weight)			
Mammary	1	0.62	O'Flaherty *et al*. (1992)/ Clewell *et al*. (2002)
Uterus	0.2	0.14	O'Flaherty *et al*. (1992)/ ICRP (1975)
Percentage of cardiac output			
Mammary	0.2	2.7	O'Flaherty *et al*. (1992)/ Clewell *et al*. (2002)
Uterus	0.5	0.62	O'Flaherty *et al*. (1992)/ ICRP (1975)

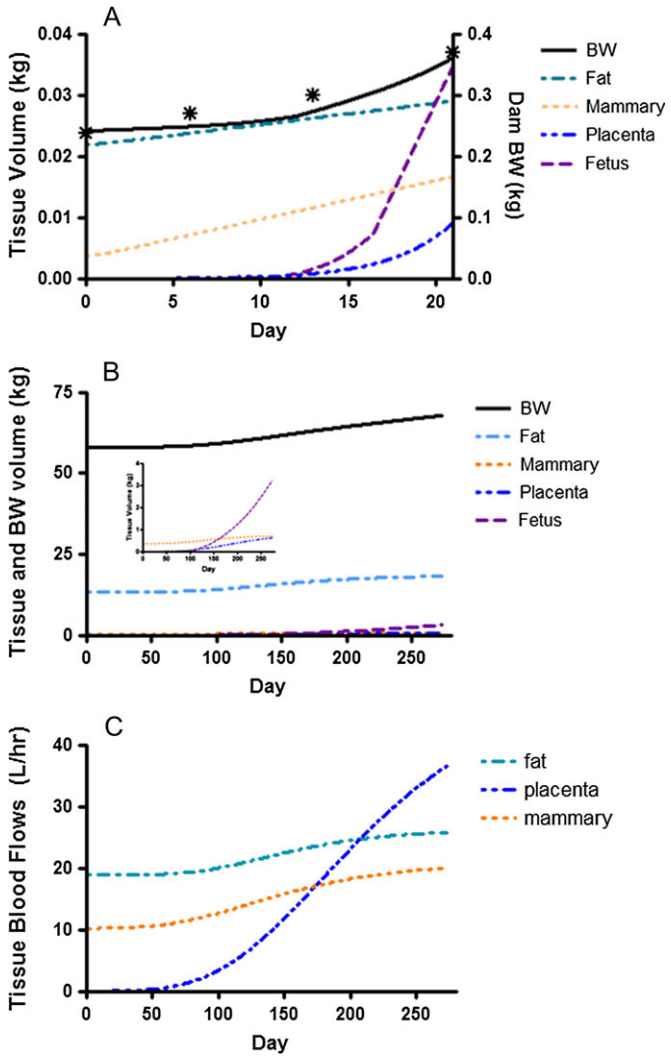

FIG. 2. Pregnancy model output showing the growth of tissues from (A) GD 0 to parturition in the rat. The asterisks indicate non–NMP-exposed dam body weights as reported in Saillenfait *et al*. (2002). (B) The tissue changes in the human model from GD 0 to parturition, the inset shows an expanded y-axis to emphasize the mammary, placenta, and fetal growth. (C) The blood flows to these tissues also were allowed to increase and, with the exception of placental blood flow, were assumed to increase proportionally to tissue volume increases, based on data from Thoresen and Wesche (1988), as described by Gentry *et al*. (2002).

TABLE 2
Initial (GD 0) Model Parameters

	Rat	Human	Source
Body weight (kg)	Variable	Variable	Individual article/ICRP(1975)
	Tissue volume (% body weight)		
Liver	3.7	3.1	Brown *et al.* (1997) and Gentry *et al.* (2002)
Rapidly perfused	7.1	4.2	Brown *et al.* (1997) and Gentry *et al.* (2002)
Slowly perfused	62.8	64.6	Brown *et al.* (1997) and Gentry *et al.* (2002)
Fat	9.0	23	Brown *et al.* (1997) and Gentry *et al.* (2002)
Skin[a]	19.0	5.1	Brown *et al.* (1997)
	Flows (l/h)		
Alveolar ventilation	4.83[b]	362.5	Brown *et al.* (1997)
Cardiac output	4.83	362.5	Brown *et al.* (1997)
	Percentage of cardiac output		
Liver	18.3	25.0	Brown *et al.* (1997) and Gentry *et al.* (2002)
Richly perfused	51.2	48.0	Brown *et al.* (1997) and Gentry *et al.* (2002)
Slowly perfused	14.0	19.0	Brown *et al.* (1997) and Gentry *et al.* (2002)
Fat	7.0	5.0	Brown *et al.* (1997) and Gentry *et al.* (2002)
Skin[a]	1.0	3.0	Brown *et al.* (1997) and Gentry *et al.* (2002)
Biochemical constants[c]			
NMP: $V_{max}C$ (mg/h/kg$^{0.75}$)	21.8	125	Optimized
NMP: K_m (mg/l)	234	141	Optimized
5-HNMP: $V_{max}C$ (mg/h/kg$^{0.75}$)	2.4	18.8	Optimized
5-HNMP: K_m (mg/l)	48	82.2	Optimized
Urinary saturable elimination			
NMP: $V_{max}C$ (mg/h/kg) (mg/h/kg)	NA	0.006	Optimized
K_m (mg/l)	NA	0.75	Optimized
5-HNMP: $V_{max}C$ (mg/h/kg) (mg/h/kg)	NA	2.02	Optimized
K_m (mg/l)	NA	19.8	Optimized
First-order urinary elimination			
K_L NMP	0.002	NA	Optimized
K_L 5-HNMP	4.9	NA	Optimized
Absorption			
Dermal liquid: K_P (cm/h)	4.7×10^{-3}	NA	Payan *et al.* (2003)
Dermal vapor: K_p (cm/h)	NA	23	Optimized
Oral: per hour	1.4	1.4	Optimized

Note. NA, not applicable, BW, body weight.

[a]Values are for total skin. The skin compartment in the PBPK model was comprised solely of the area of the skin exposured. The remainder of the skin was included in the slowly perfused compartment.

[b]Ventilation rates are body weight specific.

[c]V_{max} (mg/h)= $V_{max}C \times BW^{0.7}$.

Partition Coefficients

Partition coefficients are needed to determine tissue-specific solubility of both NMP and 5-HNMP in the PBPK model. A partition coefficient for a given chemical is the ratio of concentration achieved between two different media at equilibrium. Thus, partition coefficients are the quantitative description of a chemical's solubility in the media. N-methyl pyrrolidone (1-methyl-2-pyrrolidone) was purchased from Aldrich (> 99% pure). 5-Hydroxy-N-methyl pyrrolidone was synthesized by Ramidus AB (Lund, Sweden) and was kindly supplied by BASF (Ludwigshafen, Germany). Partition coefficients for NMP and 5-HNMP were determined in human donor blood (Golden West Biologicals, Temecula, CA). Partition coefficients were also determined from blood, liver, fat (perirenal), muscle (thigh), skin (shaved back), lung, and kidneys of male rats, from blood and liver in female rats, and from blood, liver, fetus, extraembryonic fluid, and uterus of pregnant female rats for both chemicals at the same time, using the ultrafiltration technique of Jepson *et al.* (1994). Tissues and blood were collected from a minimum of three Sprague-Dawley rats.

Preliminary studies were conducted to verify first that uptake into the tissue was stable after 18-20 h of incubation; second, that the concentrations of NMP and 5-HNMP fit within the analytically linear range; and third, that coincubation with both NMP and 5-HNMP did not alter the solubility of either chemical. After incubation, the samples were filtered using Amicon filters (MPS micropartition, 38K mw cutoff membranes; Millipore, Billerica, MA) and centrifuged for 30 min. The filtered sample (100 µl) was transferred to an high pressure liquid chromatography (HPLC) vial and analyzed using an HPLC/mass spectrometry (Agilent 100 LC/MSD, Santa Clara, CA). The sample was injected on a C18 column and eluted for 2 min with 5% aqueous acetic acid at a flow rate of 0.2 ml/min. A gradient was then run from the 1% acetic acid to a solvent containing 1% acetic acid in methanol for 8 min followed by isocratic elution with the methanol solvent for the remaining 6 min. The limit of quantitation was below 12 µg/ml and the analytical concentration curve lost linearity above 100 µg/ml, so initial concentrations of NMP and 5-HNMP in the incubation systems were targeted at 75 µg/ml.

Tissue/blood partition coefficients were calculated by dividing the measured tissue/saline coefficients by blood/saline coefficients. For the human model, tissue/blood coefficients were calculated using the rat tissue/saline coefficients divided by the measured human blood/saline coefficient.

Data for Rat Model Calibration

The pharmacokinetics of NMP has been evaluated in rats following multiple routes of exposure. Data describing the pharmacokinetics of NMP following iv exposures were used to optimize metabolic rate constants without the confounding effects of absorption. Two literature sources described iv dosing with NMP (Payan *et al*., 2002; Wells and Digenis, 1988). Wells and Digenis (1988) described the measurement of radiolabel from single iv ^{13}C-NMP exposures of 45 mg/kg in male Sprague-Dawley rats. The animals were fasted overnight, and the surgical procedure to implant the jugular cannulas was conducted just before dosing, so the rats were anesthetized with Nembutal and asleep for the first couple of hours of the exposure. The majority of urinary radioactivity was attributed to a single metabolite, which was later identified as 5-HNMP (Wells *et al*., 1992). No parent NMP or conjugated species were identified in urine. Only very minor amounts of radioactivity were recovered in feces or as exhaled CO_2.

Payan *et al*. (2002) also dosed male Sprague-Dawley rats with ^{14}C-NMP at concentrations ranging from 0.1–500 mg/kg. Unfortunately, a complete blood time course is only available for the 0.1-mg/kg dose (obtained using DigitizeIt [Share-It! Inc.; Greensburg, PA]; Fig. 1). Urinary elimination of parent NMP was noted but at low levels, suggesting a saturable process or tubular reabsorption. Urinary elimination of 5-HNMP accounted for up to 55% of the administered dose at these exposure levels.

The potential routes of interest based on developmental studies include oral, dermal, and inhalation. Since the end point of concern is change in fetal body weight following exposures to dams, data from nulliparous female Sprague-Dawley rats exposed to 10 or 100 ppm radiolabeled NMP via inhalation for 6 h were used to calibrate the inhalation route of exposure (Ghantous, 1995); the concentration of NMP in plasma was below the limit of detection for the low-exposure concentration. Two sources of oral exposure data were used to optimize an oral uptake value. Because both studies used radiolabeled NMP in a water vehicle, a single oral absorption parameter was optimized that provide the best description of both data sets (Ghantous, 1995; Midgley *et al*., 1992). Like the inhalation study, the female rat data from the report of Ghantous (1995) were used to verify the calibration of the model to this gender.

Two data sets were identified as appropriate bases for a dose-response assessment for NMP, including (1) decreases in rat fetal body weight (Saillenfait *et al*., 2003) and (2) decreases in rat pup body weight (Solomon *et al*., 1995; Staples, 1990). These two studies are summarized briefly below:

- Saillenfait *et al*. (2003)—Groups of pregnant Sprague-Dawley rats were exposed to NMP concentrations of 0, 30, 60, and 120 ppm, 6 h/day, on gestational days (GDs) 6 through 20. Dams were sacrificed on GD 21. Maternal body weights were measured on GDs 0, 6, 13, and 21; maternal food consumption was measured at weekly intervals during the gestation period. Maternal body weight gain was transiently but significantly decreased at 60 and 120 ppm on GDs 6–13 only, and maternal food consumption was reduced at 120 ppm on GDs 13–21. No significant difference in the gestational weight change, corrected for the weight of the gravid uterus, was observed at any NMP concentration. No adverse effects on embryo/fetal viability or evidence of teratogenicity were observed at any concentration tested. Fetal toxicity indicated by a 5% reduction in fetal weight was noted at 120 ppm. The study authors identified NOELs for maternal and developmental toxicity of 30 and 60 ppm, respectively. Fetal body weight data are summarized in Table 3.

- Staples (1990)/Solomon *et al*. (1995)—A two-generation reproduction study with a developmental toxicity component was conducted for NMP. For the reproduction phase, male and female rats inhaled 0, 10, 51, or 116 ppm NMP daily for 6 h/day, 7 days/week from 34 days of age to the end of the mating period for the males, and until postpartum day (PPD) 21 for the females. The only exception was that dams were not exposed to NMP from GD 20 to PPD 4. Maternal body weights were measured on GD 1, GD 21, and PPD 1, 4, 14, and 21. Dams were sacrificed after weaning on PPD 21. Reproductive parameters were not affected by NMP exposure. The protocol used by Staples (1990) could not confirm either the transient maternal body weight effects noted by Saillenfait *et al*. (2003) because gestation body weights measurements were limited to days 1 and 21 or decreased food consumption because this parameter was not measured. However, there were no dose-related changes in maternal body weight at the gestational and PPDs evaluated, consistent with the study of Saillenfait *et al*. (2003). The only potential toxicity noted in parental rats was a decrease in response to sound at 116 ppm during the premating period. For the pups, an exposure-related decrease (11%) in body weight was detected only among those whose parents both inhaled NMP at 116 ppm. This significant decrease persisted until weaning at PPD 21 when maternal exposures to NMP ceased and the dams were killed. By 28 days after birth, offspring body weights returned to control values and affected pups went on to reproduce normally. Although the decrement in pup body weight was the basis for the developmental NOEL of 51 ppm identified by the study authors, the biological significance of this transient effect is unclear. In a parallel developmental study, Staples (1990) exposed rats of both sexes to 0 or 116 ppm NMP. Females were sacrificed on GD 21 followed by fetal examination for structural alterations. Although neither dam body weight nor fetal structure was affected, parental exposure to 116 ppm NMP produced a significant decrease (7%) in fetal body weight. These data, consistent with those reported by Saillenfait *et al*. (2003) at 120 ppm, are summarized in Table 4.

TABLE 3
Fetal Body Weight Decreases in Rats Exposed to NMP (Saillenfait *et al*., 2003)

External concentration (ppm)	Internal dose (average daily AUC) (mg h/l)	Live litters	Fetal body weight (g)	
			Mean	SD
0[a]	0	321	5.67	0.34
30	94.6	20	5.62	0.36
60	193	19	5.47	0.25
120	403	25	5.39*	0.45

[a]Data for pooled historical control animals (Saillenfait *et al*., 2003, 2007).
*Significantly different from control ($p < 0.05$). Litter considered the experimental unit for statistical evaluation.

Data for Human Model

NMP is unusual in having more human pharmacokinetic data than rat pharmacokinetic data (Akesson and Jonsson, 2000; Akesson and Paulsson,

TABLE 4
Pup Body Weight Decrease in Rats Exposed to NMP (Staples, 1990; Solomon *et al*., 1995)

External concentration (ppm)	Internal dose (average daily AUC) (mg h/l)	Live litters	Pup body weight (g)	
			Mean	SD
0[a]	0	39	7.5	0.71
10.3	31.8	16	7.0	0.70
50.8	162	15	7.1	0.69
116[b]	387	37	6.9*	0.64

[a]Pooled data from two concurrent control groups.
[b]Pooled data from two concurrent high concentration test groups.
*Significantly different from control ($p < 0.05$). Litter used considered the experimental unit for statistical evaluation

1997; Akesson *et al*., 2004; Bader and van Thriel, 2006; Bader *et al*., 2005, 2007, 2008). The adult human PBPK model was calibrated with data from Bader and van Thriel (2006 and reported herein) and Bader *et al*. (2008). Prior to study start, volunteers were examined by a physician with respect to their general health status and to exclude subjects with respiratory restrictions, skin diseases, or cardiovascular diseases. The study participants were informed about the study protocol, the blood sampling procedure, and possible risks; all volunteers provided written informed consent. The study was carried out in accordance with the Helsinki Declaration, and the study protocol was approved by the ethics committee of the Leibniz Research Centre for Working Environment and Human Factors at the TU Dortmund (Germany).

Eight healthy nonsmoking male volunteers (mean age: 26 years, range: 23–29 years, wearing long pants and short-sleeved cotton shirts) were exposed whole body in groups of four to NMP vapor concentrations of 10 mg/m^3 (measured average: 9.7 ± 0.8 mg/m^3), 40 mg/m^3 (measured average: 40.3 ± 1.0 mg/m^3), and 80 mg/m^3 (measured average: 80.0 ± 1.6 mg/m^3), or approximately, 2.5, 10, and 20, respectively. Each group was exposed for 6 h and 10 min with an exposure-free interval of 10 min for collection of blood samples outside the exposure chamber. The purposely built exposure chamber (29 m^3), as described by van Thriel *et al*. (2007), was maintained at a temperature of 23°C ± 6% and a relative humidity of 39 ± 12%. The three NMP concentrations were presented in ascending order, and an exposure-free period of 1 week between subsequent sessions was strictly adhered to.

To examine the relative contribution of inhalation and dermal absorption to the total uptake of NMP during an 8-h whole-body vapor exposure, Bader *et al*. (2008) compared the excretion of NMP by human volunteers exposed to NMP vapor either with or without face shields that provided the breathing zone, mouth and nose, with filtered, NMP-free air. The dermal-only conditions were conducted with airborne NMP concentrations of 80 mg/m^3; urine data from this study was used to calibrate the model for dermal vapor uptake.

Biomonitoring

For blood sampling, an iv catheter was placed in the forearm of subjects and was used to draw all blood samples. After placing the catheter, a pre-exposure blood sample was drawn into heparin-containing disposable syringes and the first urine sample was collected in a polypropylene beaker. During each particular test day, altogether six blood samples were drawn in a separate exposure-free room (pre-exposure, 10-min break, immediately postexposure, 1-, 2-, and 4-h postexposure). Another two blood samples were collected approximately 24 and 48 h after the onset of exposure. Plasma was removed from the blood samples by centrifugation. Urine samples were collected before exposure onset, during the 10-min break, 1 and 4 h postexposure, and in varying intervals up to 48 h after the exposure onset. All samples were kept at 4°C after sampling for a maximum of 24 h. Afterward, the samples were stored at −18°C until analysis.

Analyses of urinary NMP, its metabolites, and creatinine were carried out by gas chromatography-mass spectrometry after solid-phase extraction as described by Bader *et al*. (2007). The plasma analyses were carried out in the same way with the following modifications: The analysis of NMP in plasma involved the extraction of 1 ml plasma, 50 µl internal standard (10 mg/l d_9-NMP), 1 ml toluene, and 2 ml 12M potassium hydroxide solution. For the analysis of 5-HNMP and 2-HMSI, the plasma was not dissoluted, but only 0.5 ml were used (+ 50 µl internal standard; 500 mg/l d_4-5-HNMP and d_3-2-HMSI). The final volume after the sample elution from the solid-phase extraction cartridges and subsequent evaporation of the solvent was 0.1 ml.

Under these conditions, the limits of determination as calculated by the German DIN 32 645 (German Institute for Standardization) were 10 µg/l for NMP, 0.4 mg/l for 5-HNMP, and 0.2 mg/l for 2-HMSI. The interassay imprecision was 5% for NMP (c = 400 µg/l, n = 10), 1% for 5-HNMP (c = 15 mg/l, n = 10), and 7% for 2-HMSI (c = 7.5 mg/l, n = 10).

For quality control purposes, pooled urine and plasma samples were prepared from nonexposed individuals. The quality control material for urine analyses was spiked at concentrations of 200 µg/l NMP, 20 and 150 mg/l 5-HNMP, and 10 and 75 mg/l 2-HMSI. In plasma, the spikes were 400 µg/l NMP, 15 mg/l 5-HNMP, and 7.5 mg/l 2-HMSI. The quality control material was divided into aliquots and stored together with the study samples at −27°C. Quality control samples were included in every analytical series to verify the stability of the biomonitoring methods throughout the study. No external quality control material is currently available for NMP or its metabolites in plasma or for NMP in urine. The accuracy of the analysis of urinary 5-HNMP and 2-HMSI was certified by successful participation in the German External Quality Assurance Scheme (Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin, c/o Institute of Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg, Germany) during the study.

Other Human Exposure Data

After the model was calibrated to the data above, the human exposure data of Akesson and Paulsson (1997) were used to validate the model. Blood and urine were collected from six male human volunteers to determine the pharmacokinetics of NMP following exposures to 10, 24, or 53 mg NMP/m^3 (2.5, 5.9, and 13 ppm) in a whole-body exposure chamber. Participants performed light work while in the chamber and reported no discomfort from these exposures.

Finally, the biomonitoring data described by Xiaofei *et al*. (2000) were used to validate the model fit to real-world exposures to low levels of NMP vapor. In this study, workers washing lenses with liquid NMP and volunteers observing these activities were exposed to NMP for 12 (5 days per week) and 9 h (1 day only), respectively, with a 1-h break after 4 h. Activated charcoal samplers were used to estimate personal exposures to NMP vapor. NMP was measured in the plasma of the workers and volunteers at predetermined intervals. Plasma NMP levels and blood sampling times were quantitated from Figure 3 using DigitizeIt (Share-It! Inc.). When digitized sample times differed significantly (≥ 1 h) from times stated in the text, preference was given to times indicated in Figure 3 of Xiaofei *et al*. (2000). Simulations assumed 5800 cm^2 of skin surface area were exposed to NMP vapor and that workers were either resting or performing light (50 watt) work. Although workers wore gloves and aprons to prevent direct contact with liquid NMP, one worker was treated for dermatitis due to direct contact with NMP, suggesting that workers may have occasionally experienced dermal exposures to both NMP vapor and liquid.

BMD Modeling

All dose-response modeling was conducted using the U.S. Environmental Protection Agency's (USEPA) Benchmark Dose Software (BMDS, version 2.1) and in a manner that is consistent with USEPA benchmark dose guidelines (USEPA, 2000). The data were assessed in terms of an internal dose measure, based upon the area under the curve (AUC) for parent chemical in maternal blood, averaged over the days of exposure during gestation as predicted by the rat PBPK model. Four dose-response models for continuous data (linear, polynomial, power, Hill) were fit to arithmetic means for body weight for the two dose-response data sets (Tables 3 and 4). A benchmark response rate (BMR) of 1 SD for unexposed animals serves as the default value for continuous data sets, in the same way that a 10% BMR serves as the default value for dichotomous data set (USEPA, 2000), and was selected for this assessment. A BMR of 1 SD has been used in deriving reference values for a number of chemicals, including phenol, benzene, cyclohexane, toluene, n-hexane, pentabromodiphenyl ether, tetrabromodiphenyl ether, nitrobenzene, and ethyl tertiary butyl ether (IRIS, 2009). The dose-response models were used to predict the concentration resulting in a decrease in mean body weight corresponding to 1 SD (BMC1SD) and its 95% lower confidence limit (BMCL1SD). A best fitting model was identified by visual inspection, Akaike's information criteria, goodness-of-fit p value, and model uncertainty (using the ratio of the BMC:BMCL, in which a smaller value indicates less model uncertainty). Because the nested models available from BMDS are for dichotomous data (i.e., incidence) only and because the maternal effects of NMP during gestation are mild and transient in nature (Saillenfait *et al*., 2003), a nested model that would account for potential litter effects was not used with the continuous data sets described here.

Additional considerations in modeling the Saillenfait *et al*. (2003) data include the following. Because historical control data are available for this end

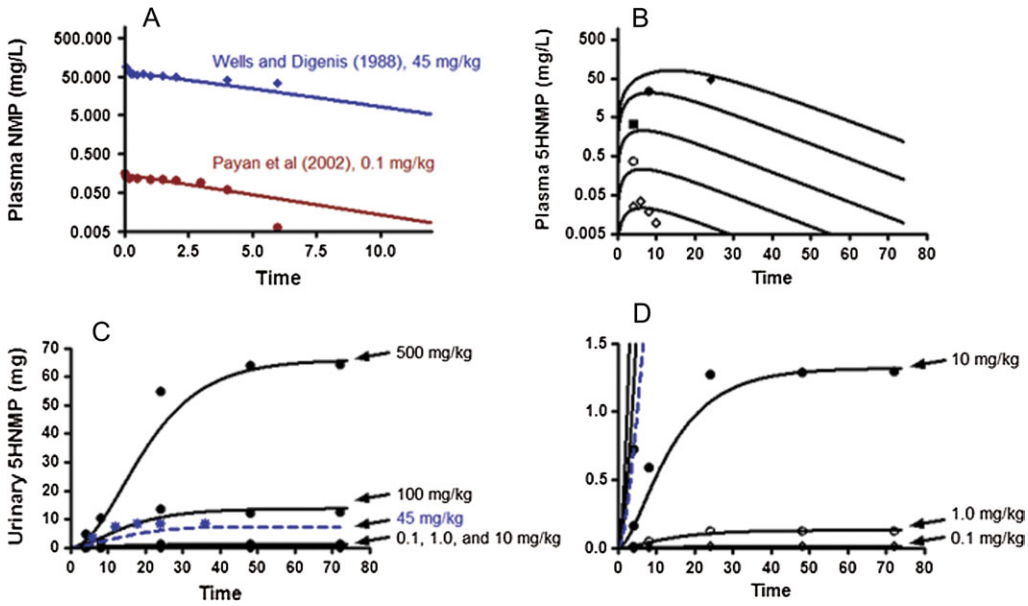

FIG. 3. Fits of the model to iv data from Payan *et al.* (2002) and Wells and Digenis (1988). (A) Plasma NMP, (B) Plasma 5-HNMP (from Payan *et al.*, 2002, only), (C) Urinary 5-HNMP. The dashed line represents data from Wells and Digenis (1988). (D) Expanded y-axis from (C) to show fits to 0.1 to 10 mg/kg exposures.

point, these data were used to provide a more complete description of variation in unexposed animals. Use of historical control data is consistent with USEPA guidelines for BMD (USEPA, 2000). In addition, a homogeneous variance model was assumed for these data despite slight differences in the SDs noted for body weight in the two highest test groups (Table 3). A nonhomogeneous variance model was not adopted based upon a consideration of the following: (1) inspection of the historical control data indicates that the variation in the two highest test groups falls within the range of variances reported in historical controls (0.20–0.56) and (2) a simple power model used by BMDS would not adequately describe the non-monotonic change in variance with dose. The difference in SD values for the two highest treatment groups is likely to be a random effect, rather than an effect of treatment.

With respect to modeling the Staples (1990) data, the following considerations were included in the analysis. Because the study design included two concurrent control groups, these were combined for the purposes of dose-response modeling. In addition, three groups of animals were exposed to the highest test concentration (116 ppm): (1) exposed female rats mated with exposed male rats, (2) exposed females rats mated with unexposed male rats, and (3) unexposed female rats mated with exposed male rats. Because no treatment-related effects on pup body weight were observed in the male-only exposed groups, only exposure to the dams was considered to be relevant to the changes in pup body weight. For this reason, the results for two concurrent test groups of females exposed to the highest concentration were combined together for the purposes of dose-response modeling (Table 4).

The resulting BMC values expressed in terms of rat internal dose (AUC) were converted to corresponding HEC using the human PBPK model developed for NMP. HECs were defined to correspond to a typical occupational exposure scenario (8 h/day, 5 days/week) and included both an inhalation and a dermal absorption component as described in the "Results" section.

RESULTS

Partition Coefficients

The tissues most likely to display changes during pregnancy are blood, liver, and fat (Corley *et al.*, 2003). Differences in blood partitioning are often most notable between genders. Since all other partition coefficients are calculated relative to the blood/saline partition coefficient, this value is the most important to assess. Solvents, with structures similar to NMP are generally soluble in blood and tissues and, therefore, have blood:tissue partition coefficients near unity. The saline:tissue partition coefficients for NMP in all tissues measured range from 0.34 to 4.25. Saline:tissue partition coefficients for 5-HNMP are a little higher than for NMP, indicating that 5-HNMP is slightly more soluble in the tissues than in saline (Table 5).

Non-Pregnant Rat Model Calibration

The studies of Wells and Digenis (1988) and Payan *et al.* (2002) were used to calibrate the model by iteratively fitting the data of Payan and verifying the best possible fit to the data of Wells and Digenis. The model fits to the plasma NMP concentrations and urinary 5-HNMP from both studies, and the fits to the 5-HNMP plasma concentrations from Payan *et al.* (2002) are shown in Figure 3. Despite the differences in exposure concentrations and other laboratory differences, the model adequately describes both data sets.

Since the NOEC is derived from inhalation exposures of female rats, the fit of the model to the inhalation data of Ghantous (1995) is shown in Figure 4. A bioavailability of 100% of alveolar ventilation (alveolar ventilation [QPC] is calculated as 2/3 total ventilation) was assumed for inhalation exposures. In order to also compare internal dose metrics for oral routes of exposure from the gavage and feed studies, the model was also fit to oral pharmacokinetic data (Ghantous, 1995; Midgley *et al.*, 1992). The model fits to the plasma NMP concentrations from these two studies are shown in Figure 5.

TABLE 5
Tissue:Blood Partition Coefficients (Mean, SD [*N*])

Tissue	Partition coefficient	
	NMP	5-HNMP
Male rat		
Blood:saline	3.43 ± 1.13 (6)	5.07 ± 2.41 (6)
Muscle:saline	1.96 ± 0.50 (6)	9.2 ± 3.74 (6)
Fat:saline	2.10 ± 1.64 (6)	2.02 ± 1.29 (6)
Liver:saline	3.49 ± 0.90 (5)	15.5 ± 4.60 (6)
Kidney:saline	3.98 ± 1.61 (6)	40.5 ± 10.9 (6)
Skin:saline	0.42 ± 0.27 (10)	1.30 ± 0.5 (10)
Lung:saline	0.44 ± 0.22 (6)	2.67 ± 0.89 (6)
Female		
Blood:saline	2.29 ± 1.23 (10)	5.66 ± 1.83 (6)
Liver:saline	2.65 ± 0.92 (6)	30.9 ± 3.57 (6)
Pregnant female rat		
Blood:saline	1.92 ± 0.82 (10)	2.08 ± 0.82 (10)
Liver:saline	2.57 ± 0.85 (6)	28.7 ± 7.01 (6)
Fetus:saline	1.06 ± 0.26 (5)	5.41 ± 2.64 (5)
Extraembryonic fluid:saline	2.64 ± 1.18 (6)	4.25 ± 2.08 (6)
Uterus:saline	0.34 ± 0.19 (6)	4.07 ± 0.72 (6)
Human		
Blood:saline	4.25 ± 1.28 (7)	6.19 ± 0.89 (7)

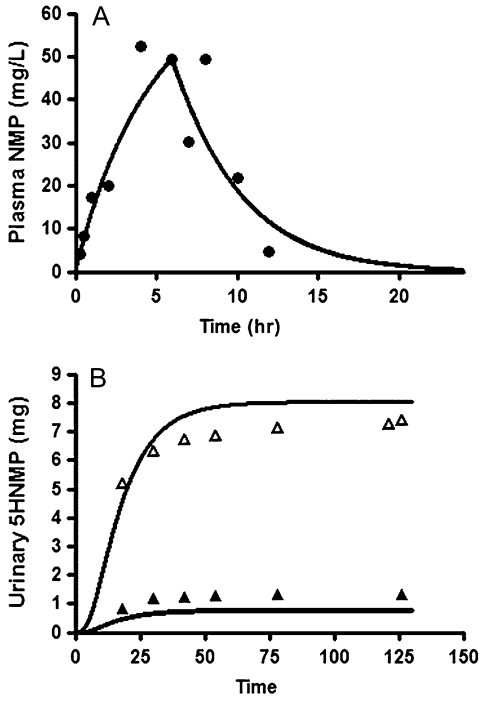

FIG. 4. Fits of the model to inhalation data of Ghantous (1995). (A) Plasma NMP from a 100-ppm exposure (plasma NMP from a 10-ppm exposure were below quantitation levels). (B) Urinary elimination of 5-HNMP in female rats exposed to both 10 and 100 ppm.

Human Dosimetrics

The adult human PBPK model was calibrated using blood and/or urine data collected from volunteers receiving either whole-body (Bader and van Thriel, 2006; described herein) or dermal-only exposures to NMP vapor (Bader *et al.*, 2008). Data from the dermal-only and whole-body studies were used iteratively to optimize metabolic rate constants and the dermal absorption of NMP vapor (Table 2). Total body surface area was calculated based on the equation of Dubois and Dubois as reported in ICRP (1975). Vapors were assumed to access both bare skin and additional surface area under sleeves, and the exposed skin surface was assumed to be 35% of the total skin surface area. The optimized K_P for the dermal absorption of NMP vapors is 23 cm/h—which is ~5000 times higher than the rate for absorption of NMP as a liquid. This dermal uptake paradigm is consistent with other low vapor pressure solvents like the glycol ethers (Corley *et al.*, 1997). The fits of the model to the 10, 40, and 80 mg/m^3 dual inhalation and dermal exposures are shown in Figures 6A–D. The elimination of 5HNMP in these human subjects is proportionally much higher than observed in rats, which is reflected in the higher rates of metabolism in humans (Table 2).

A sensitivity analysis was conducted in acslXtreme to identify the importance of key parameters on estimates of blood NMP. The analysis measured the change in model output corresponding to a 1% change in a given model parameter when all other parameters were held fixed. A normalized sensitivity coefficient of 1 indicates that there is a one-to-one relationship between the fractional change in the parameter and model output; values close to 0 indicate a small effect on model output. A positive value for the normalized sensitivity coefficient indicates that the output and the corresponding model parameter are directly related and a negative value indicates they are inversely related. Sensitivity coefficients at the end of an 8-h exposure for cardiac output, pulmonary ventilation, and K_P were −0.27, 0.62, and 0.35, respectively.

After calibrating the model using the data from the studies described in this article, the fits of the model to other published human pharmacokinetic data were assessed. In the studies described by Akesson and Paulsson (1997), six male human male volunteers were exposed to concentrations of 10, 24, and 53 mg/m^3 (2.5, 5.9, and 13.1 ppm) separated by 2 weeks. The exposure was stopped for 5 min of sample collection at 2, 4, and 6 h—this was not accounted for in the model. Data for the urinary elimination of 5-HNMP was given in micromoles per liter and millimoles per mole creatinine basis. Since the volume of elimination was unknown, standard creatinine clearance values (Tietz, 1995) were used to estimate the amount excreted. The volunteers were described as doing light work, so physiological parameters for a 50-watt work standard were used in the model (Jonsson *et al.*, 2001). The fit of the model to the urinary elimination of 5-HNMP and plasma NMP predictions are exceptional (Figs. 6E–F).

Finally, the biomonitoring study of Xiaofei *et al.* (2000) was used to determine the fits of the model to workplace exposures. In this study, blood levels of NMP were monitored in workers

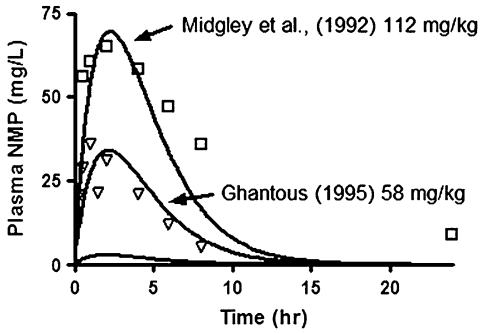

FIG. 5. Fits of the model to oral data of Ghantous (1995) and Midgley *et al*. (1992) showing plasma NMP concentrations.

and observers exposed to NMP for 12 h/day (5 days per week) and 9 h/day (1 day only), respectively, during a workweek. The graphs in Xiaofei *et al*. (2000) show predicted exposures for all 5 days and indicate the highest daily exposures for the workers occurred on days 1 and 5 for all the workers except possibly Worker C who may have had a slightly higher exposure on day 4; therefore, the high dosimeter data were used to model these exposures, with and without increased ventilation and heart rates describing light work (Jonsson *et al*., 2001). Despite the use of protective clothing, it was reported that one worker developed dermatitis after direct contact with liquid NMP, suggesting that NMP blood levels in workers may reflect some contribution from dermal contact with liquid NMP. Notwithstanding this caveat, the NMP blood levels in workers and observers predicted by the PBPK model based on external NMP exposures measured with personal dosimeters is exceptional (Fig. 7).

Pregnant Model

Based on studies in rats, developmental toxicity, measured as a reduction in fetal/pup body weight, has been identified as the end point of concern for NMP by all routes of exposure examined (Becci *et al*., 1982; Lee *et al*., 1987; Saillenfait *et al*.,

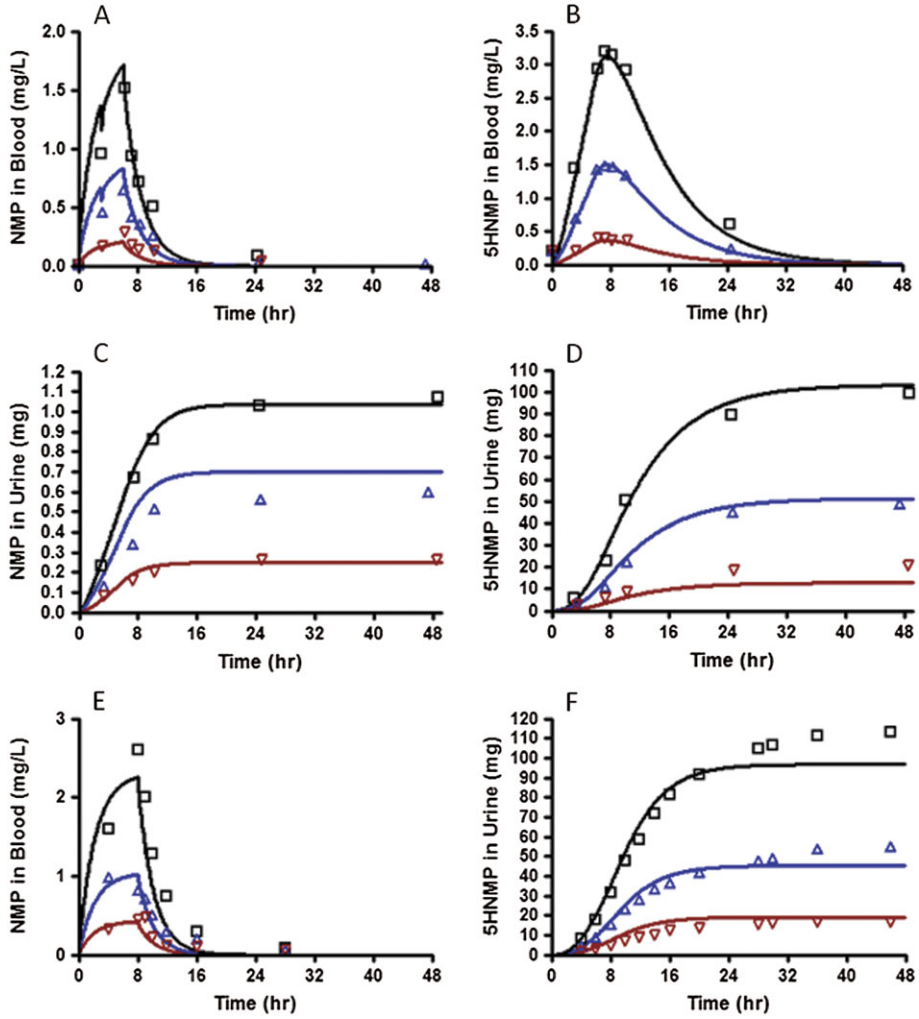

FIG. 6. Fits of the model to human inhalation data from this study: (A) plasma NMP, (B) plasma 5-HNMP (C) urinary NMP, and (D) urinary 5-HNMP. Model fits to the published data of Akesson and Paulsson (1997) are shown in (E) and (F).

2002, 2003, 2007a,b; Thornton, 1999). Rat and human pregnancy PBPK models of NMP were developed to extrapolate internal dose metrics from the pregnant rat studies to determine potential dose metrics for pregnant women. The pregnancy model was calibrated to the data presented in Figures 3–5, on GD 1, and the fits to the pregnant and non-pregnant models were essentially the same (data not shown). The placental compartment includes flow-limited kinetics as part of the dam, with a first-order transfer rate, between the placenta and fetal blood compartment. Placental blood flow was simulated based on the time elapsed since the beginning of gestation (Gentry *et al*., 2002), and a placental area factor (PAF) was used to describe transfer of NMP between the maternal and fetal compartments (Gentry *et al*., 2002; O'Flaherty, 1994). In the absence of data to calibrate the PAF, a maximum transfer rate, which essentially results in blood flow–limited kinetics between maternal and fetal compartments, was used (0.1 per hour, data not shown). The equations used to describe the placental transfer of NMP are given below (eq. 1–3).

Equation 1: Change in amount of chemical in placental compartment (mg/h).

$$\mathrm{dAPla}/\mathrm{d}t = (\mathrm{QPLax}(\mathrm{Cart} - \mathrm{CVPLa})) + \mathrm{PAF}(\mathrm{Cfet} - \mathrm{Cpla})). \quad (1)$$

Equation 2: Change in amount of chemical in fetal tissue compartment (mg/h).

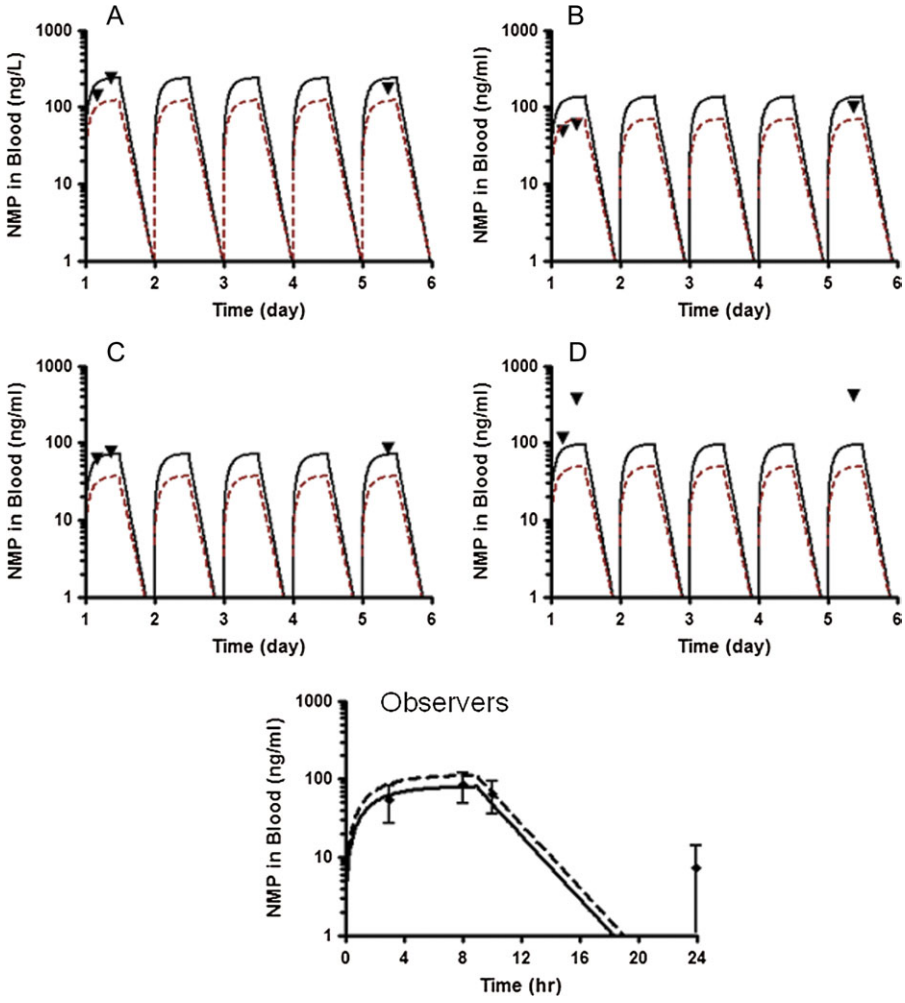

FIG. 7. Fit of the model to the workplace exposure data of Xiaofei *et al*. (2000), showing the model-predicted average blood concentrations for the 5-day workweek biomonitoring study. Xiaofei *et al*. (2000), reported blood concentrations in these subjects only on the first day at 4 and 8 h of exposure and on the last day at 8 h of exposure (workers A–D). Model predictions were derived from the reported 5-day average exposures estimated via dosimetry under conditions of light work (solid lines) or rest (dashed lines). One subject was reported to have dermatitis from direct contact to liquid NMP. The observers watched the workers for 9 h, and blood was measured at 3 and 8 h during exposure and 1 and 15 h after exposure. Although the 24-h time point (15 h after the end of exposure) is included for completeness, in the figures in the article by Xiaofei *et al*., these data are at or below the reported limit of detection. For the observers, the solid line shows the model output from the lowest exposure estimates and the dashed line is based on the highest dosimeter estimates.

$$\frac{\mathrm{dAFet}}{\mathrm{d}t} = \mathrm{PAD}(\mathrm{CPla} - \mathrm{Cfet}). \quad (2)$$

Equation 3: Scaling of diffusion constant (l/h).

$$\mathrm{PAF} = \mathrm{PAFC} \times \mathrm{Vfet}^{0.7s}. \quad (3)$$

Where Afet is the amount of chemical in fetal compartment (mg), APla is the amount of chemical in placental compartment (mg), Cart is the concentration of chemical in arterial blood (mg/l), Cfet is the concentration of chemical in fetal tissue (mg/l), Cpla is the concentration of chemical in placental tissue (mg/l), CVPla is the concentration of chemical in venous blood leaving placenta (mg/l), QPla is the current (GD specific) scaled blood flow to the placenta during pregnancy (l/h), and VFet is the current scaled volume of fetal tissue during pregnancy (l).

To estimate internal dose metrics for the rat, the pregnancy model was used to duplicate the exposure patterns associated with a NOEL or LOEL from each of the key developmental studies. The rat pregnancy model was used to simulate the study-specific exposure. At the end of each simulation, the average daily AUC or the maximum blood concentration (C_{max}) for maternal blood was determined. The maternal blood AUC did not vary greatly over the course of gestation, for example, the model-predicted AUC for the 60-ppm exposure from the inhalation studies described by Saillenfait *et al.* (2002) were 189 and 195 mg h/l for non-pregnant and end of gestation, respectively. The maternal blood AUC was also not sensitive to the choice of placental transfer (PAF) (sensitivity analysis conducted under the conditions of rat NOEL from Saillenfait *et al.* [2002] using acslXtreme, sensitivity coefficient was $< 1 \times 10^{-8}$) since the fetal compartment was very small compared to maternal blood. The fetal AUC and C_{max} were linearly proportionate to maternal exposure. In the absence of fetal validation data, it was decided that fetal internal dose measures are too uncertain for use in the assessment and that the maternal measures, which are better validated (see Figs. 3–5), are a more acceptable dose metric to apply to the BMD analysis.

An average daily AUC for each internal dose metric was then calculated as the total AUC for the dose metric divided by the length of exposure (number of days) during pregnancy (Table 6). An example model output showing 60-ppm inhalation exposures in rats is shown in Figure 8A. Figure 8B shows a detail output (representing the sixth month of gestation) of the human model when a 58-kg (at GD 0) woman is exposed to 480 ppm NMP vapor. For the human simulations, exposures were considered to include both inhalation uptake and dermal uptake and were to an 8-h workday, 5 days/week. The comparison of C_{max} and AUC species differences also justified the use of maternal AUC as the dose metric since human fetal concentrations were comparatively lower than rat fetal concentrations, making the use of the maternal blood metric more conservative (compare Figs. 8A and 8B).

Simulations were run describing the rat gavage exposures described in Saillenfait *et al.* (2002) and the dermal exposures described by Becci *et al.* (1982). Additionally, a first-order oral absorption rate was used to estimate a feed study where rats primarily ate overnight (oral absorption rate 0.4 per hour) to estimate the affect of this pseudo-infusion on plasma NMP AUC and mimic the two-generation diet bioassay (Thornton, 1999). This study was included to determine if the predicted AUC from a two-generation diet reproduction study might be similar to the two-generation inhalation reproduction study of Staples *et al.* (1990). Reduced fetal body weights were observed in all these studies, and the model was used to compare the NOEL and LOEL internal NMP blood AUC following these different routes of exposures (Table 6).

BMD Results

The geometric mean of the pup and fetal weights from Saillenfait *et al.* (2002) and Staples (1990) were compared and considered appropriate metrics since the observations in these two studies were made within the same sex, species, and strain of rat. Furthermore, observations for the two data sets are very close in time (GD 21 vs. postnatal day 1, or ~24 h apart). Since there was no lactational exposure of pups to NMP that could have contributed to the effects on pups weights in the Staples (1990) study, the two data sets are equivalent from a gestational exposure perspective. The dose-response trends observed for body weight in these two studies are essentially the same, as evidenced by nearly identical BMD values (see Table 7).

For the two key dose-response data sets, the Hill model failed to generate BMDL values and, therefore, was not considered useful. Of the three remaining models, the linear model provided slightly better fits based upon the criteria cited, and all three models predicted very similar results (within approximately 5%; data not shown). A linear model was considered to provide the best overall fit to both data sets (p value = 0.718 for the Saillenfait data, 0.123 for the Staples data set; Fig. 9). The linear model predicted very similar results from the two data sets for the BMC1SD (470 and 540 mg h/l, expressed in terms of rat internal dose, rounded to two significant figures) and for the BMCL1SD (340 and 360 mg h/l) (Table 7). The geometric mean values for the two studies are calculated to be 500 mg h/l for the BMC1SD and 350 mg h/l for the BMCL1SD. Using the human PBPK model developed for NMP, the geometric mean values correspond to respective HEC of 700 and 480 ppm (occupational exposure of 8 h/day, 5 days/week) (Table 7). With the application of appropriate UF, these HEC can serve as a basis for establishing an OEL for NMP.

DISCUSSION

This PBPK model was shown to predict NMP blood levels following various exposure scenarios in rats. The model was

TABLE 6
Key Developmental Toxicity Studies

Route	Days (GD)	End point	Reference	NOEL/LOEL	24 h AUC (μg h/ml) NOEL	24 h AUC (μg h/ml) LOEL	C_{max} (mg/l) NOEL	C_{max} (mg/l) LOEL
Gavage	6–20	Fetal BW	Saillenfait *et al.* (2002)	125/250 mg/kg/day	944	2190	113	238
Dermal	6–16	Fetal BW	Becci *et al.* (1982)	237/750 mg/day	941	1600	81.3	103
Feed	0–20	Newborn BW	Thornton (1999)	160/350 mg/kg/day	1110	2840	NA	NA
Inhalation	6–20	Fetal BW	Saillenfait *et al.* (2003)	60/120 ppm/6 h/day	193	403	25.3	52.3
Inhalation	0–20	Newborn BW	Solomon *et al.* (1995)	50.8/116 ppm 6 h/day	162	387	21.1	49.8

Note. BW, body weight; NA, because animals are assumed to consume dosed feed via a first-order process over 12 h, a reliable C_{max} cannot be determined.

developed to facilitate extrapolation of pharmacokinetic data from rats to humans to improve on a health risk assessment for NMP and to provide a basis for setting an OEL. Effects in non-pregnant adult animals are only seen after relatively high-dose exposures. The most sensitive toxic end point is the reduced fetal/pup body weight seen after oral, dermal, and inhalation exposures to NMP (see Table 6). The internal dose metrics from the inhalation studies are significantly lower than those from the oral and dermal exposure studies. Thus, this end point and this route are very conservative. Since fetal pharmacokinetic data were unavailable, maternal blood NMP AUC was used as the dose metric for interspecies extrapolation. In rat whole-embryo culture, the NMP parent compound was shown to have the most embryotoxic effects (Flick *et al*., 2009). The parent compound was also noted by Saillenfait *et al*. (2007) to be the most developmentally toxic form of NMP. Thus, the blood concentration of the parent compound was the internal dose metric of interest for these comparisons. The model includes repeated daily exposures via inhalation as well as the oral and dermal routes; rat body weights and other exposure-specific parameters were set to reflect the study protocols outlined in Table 6. It is interesting to note that the lowest blood NMP AUCs were associated with the inhalation NOELs reported by Solomon *et al*. (1995) and Saillenfait *et al*. (2003). In fact, the NOEL from the oral (both gavage and diet) and dermal studies (Becci *et al*., 1982; Saillenfait *et al*., 2002; Thornton, 1999) resulted in predicted AUCs that were higher than the LOEL AUCs from the inhalation studies. One possible explanation is that maternal stress, from inhalation of an irritant chemical at near saturation, combined with a subthreshold level of NMP was sufficient to produce decreases in pup body weight. Support for this contention is found in the inhalation studies where shortly after the start of exposure, transient maternal toxicity was noted by Solomon *et al*. (1995) (decrease response to sound only during premating at 116 ppm) and Saillenfait *et al*. (2003) (decrease in dam body weight gain only during the first week of exposure at 60 and 120 ppm). A human correlate for this transient response may be the rapid (4 h) adaption of olfactory sensations reported by volunteers inhaling NMP at non-irritating concentrations ($\leq$ 20 ppm) for up to 8 h (van Thriel *et al*., 2007).

A number of decisions were made in the dose-response assessment that affected the resulting BMD estimates. The assessment presented here relied upon a PBPK-derived internal dose measure (i.e., AUC for parent NMP). Use of rat and human PBPK models for NMP resulted in BMC human equivalent values that were considerably larger (~4.6-fold) than would be obtained using rat external concentration (ppm) as the dose measure. For both data sets, the power model returned BMC values that were identical to those obtained using the linear model, while the Hill and polynomial models failed to return reliable results. A BMR of 1 SD was used in this

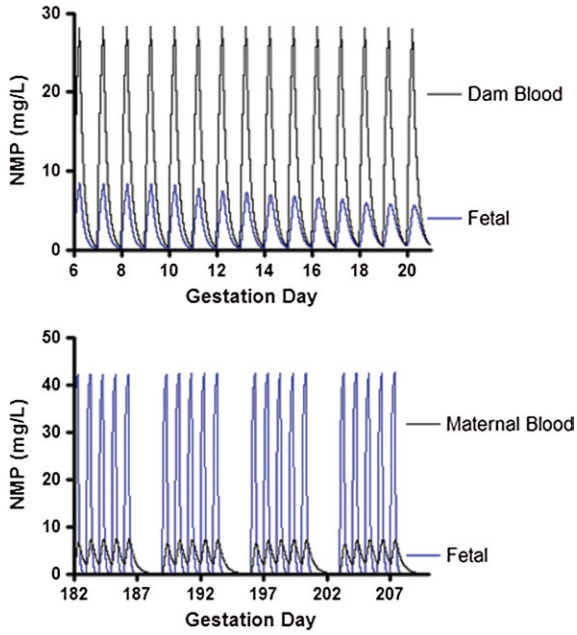

FIG. 8. Model simulations of daily inhalation exposures in (A) rats exposed to 60 ppm/day over the course of gestation (as described in Saillenfait *et al*., 2003) and (B) humans exposed in a workplace scenario (5 days/week) over the course of gestation. Human exposures include dermal absorption, and the x-axis was compressed to show only month 6 as an example.

TABLE 7
BMC Values[a] for NMP Based upon the Staples (1990) and Saillenfait *et al.* (2003) Data Sets for Fetal/Pup Body Weight

	Internal dose (average daily AUC, mg h/l)		HEC (ppm, 8 h/day, 5 days/week)	
Data set	BMC1SD[a]	BMCL1SD	BMC1SD	BMCL1SD
Saillenfait *et al.* (2003)	470	340	650	470
Staples (1990)/ Solomon *et al.* (1995)	540	360	750	500
Geometric mean	*500*	*350*[b]	***700***	***480***

Note. The values in bold/italic indicate not applicable.
[a]BMC values rounded to two significant figures.
[b]The PBPK model indicates a dose of 105 ppm (6 h/day/21 days of gestation, as described by Staples, 1990) results in an average 24 h AUC of 350 mg h/l in rats.

dose-response assessment (USEPA, 2000). Use of alternative BMR values of 5% or 10% for continuous data sets would result in moderately lower (use of 5%) or higher (use of 10%) BMC1SD values but are within a factor of 2 of the BMC1SD values used here.

With respect to data set selection, a decision was made to rely upon both the Staples (1990) and Saillenfait *et al.* (2003) data sets. The resulting geometric mean BMC1SD estimate is slightly higher (< 10%) than was estimated for the Saillenfait data set alone and slightly lower (< 10%) than was estimated for the Staples data set alone. The pooling of historical control data for the Saillenfait data set had negligible impact upon the BMC1SD, but served to tighten the range between the BMC1SD and BMCL1SD, resulting in a slightly higher (< 15%) BMCL1SD value. This result is consistent with reduced uncertainty in the BMC estimates as a result of including more information in the model. The pooling of data (concurrent control and high concentration group) for the Staples data set resulted in BMC1SD and BMCL1SD values that were moderately higher (~30–35%) than without pooling these dose groups.

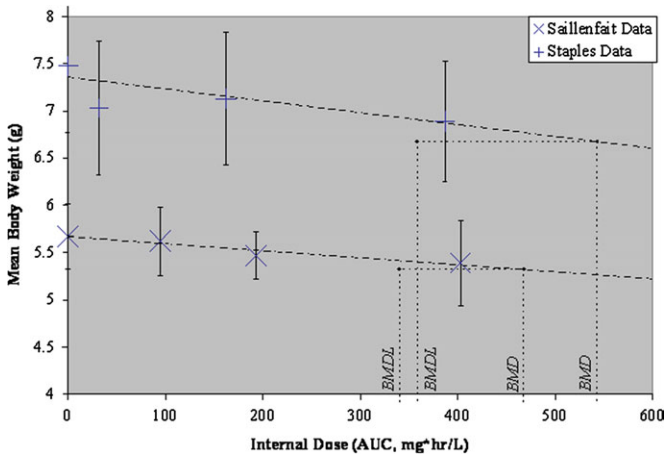

FIG. 9. Linear BMD model fit to dose-response data for body weight changes in developing rats, data from Saillenfait *et al.* (2003) and Staples (1990)/Solomon *et al.* (1995).

The selection of fetal/pup body weight as the critical end point for this assessment is a conservative, health-protective decision. The rat PBPK model indicates that the internal dose metric of 350 mg h/l corresponds to an NMP vapor concentration of 105 ppm. This concentration approximates the highest concentration (100 ppm) used in a 2-year, whole-body, inhalation study in rats where a slight (6%) decrement in male body weight was the only effect noted (Lee *et al.*, 1987) and is below the concentration of 116 ppm reported by Staples (1990) to result in decreased pup body weight. The latter effect does not appear to be biologically significant since the effect was transient (i.e., pup body weights returned to the control level 1 week after terminating maternal inhalation exposures) and the affected pups subsequently went on to reproduce normally.

The use of a PBPK model to quantitatively describe physiological differences between rats and humans reduces the uncertainty in species extrapolations and should thereby serve to supplant traditional UFs, which are used when limited data are available. Since NMP has been extensively studied, especially in humans, there were a number of rat and human data sets available to validate the model use in both rats and humans. Of the many categories of UF, the use of the PBPK model reduces, or eliminates, the need for an UF describing uncertainty in interspecies extrapolation. The use of the PBPK model does not, however, replace the interhuman variability UF. The HEC determined in this study is ~50 times higher than the OELs (10 ppm) derived by the American Industrial Hygiene Association and SCOEL (2007), indicating that the more recently enacted international OELs currently in effect are sufficient to protect workers.

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