J Occup Health 2000; 42: 321–327

Journal of Occupational Health

A Linear Pharmacokinetic Model Predicts Usefulness of N-Methyl-2-Pyrrolidone (NMP) in Plasma or Urine as a Biomarker for Biological Monitoring for NMP Exposure

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Abstract: A Linear Pharmacokinetic Model Predicts Usefulness of N-Methyl-2-Pyrrolidone (NMP) in Plasma or Urine as a Biomarker for Biological Monitoring for NMP Exposure: Xiaofei E, *et al.* Department of Hygiene, Akita University School of Medicine—N-methyl-2-pyrrolidone (NMP: C_5H_9NO:CAS number 872-50-4) is an increasingly used solvent due to the lack of ozone depleting activity. The aim of this study is to construct a simple pharmacokinetic model for NMP. In factory A, four workers who were exposed to NMP at 0.09–0.69 ppm for 12 h by time weighted average (TWA) were followed up for an entire workweek. Their NMP concentrations in plasma and urine were monitored during the observation period. Five volunteers were exposed to NMP during the observation of workers in the factory A for eight hours. NMP kinetics in plasma and urine were monitored for 2 d after exposure. Concentrations of NMP in plasma and urine as standardized by creatinine concentrations were used to construct a one compartment pharmacokinetic model. The model successfully simulated the kinetics in four workers and five volunteers. In the next step, the model was applied to eight workers in another factory: they were exposed to NMP for 12 h at 0.04 to 0.59 ppm by TWA. The model could successfully predict kinetics of NMP levels in plasma and urine at the end of work. The model was then applied to experimental exposure cases in the literature. The model successfully predicted the concentrations of NMP in plasma and urine at the exposure intensity level of 12 ppm $\times$ 8 h. These results imply that metabolic saturation does not occur up to the exposure intensity of 12 ppm $\times$ 8 h and demonstrate the usefulness of determinations of NMP in plasma and urine for biological monitoring.
(J Occup Health 2000; 42: 321–327)

Key words: N-methyl-2-pyrrolidine, Pharmacokinetic modeling, Biological modeling

Received Aug 7, 2000; Accepted Sept 18, 2000
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N-methyl-2-pyrrolidone (NMP: C_5H_9NO:CAS number 872-50-4) is an increasingly used solvent. There have been many commercial uses for it in the petrochemical industry as an extraction agent, in the microelectronic fabrication industry, and in the fine chemical industry as a synthetic material or a catalytic agent. In pharmaceutical applications, NMP is used as a vehicle for topically applied drugs to facilitate absorption into the skin.

Chlorinated solvents such as methylene chloride, 1,1,1-tricholoroethane, tetrachloroethylene and tricholoroethylene, have been widely used as degreasing agents in various occupational settings. Recently, the ban on chlorinated organic solvents due to their ozone depleting activity (Montreal Protocol, 1987) accelerated the introduction of alternate solvents. NMP is one such solvent, and is now being increasingly introduced in many developed countries.

Animals studies showed that NMP is readily absorbed through direct skin contact[1)], or by inhalation[2)] or by ingestion[1)]. They also showed that NMP has low organ toxicity. Several studies, however, indicated that it has reproductive and developmental toxicities[3–5)]. In humans, information on the toxicity of NMP is fragmentary. One report describes severe dermatitis upon prolonged contact[6)]. Workers in Japan exposed to NMP were followed up over 2 yr and were found to have increases in the hemoglobin concentration and the number of leukocytes in peripheral blood as well as periodic increases in GOT and GPT[7)]. Still another report

describes a case of stillbirth in a patient who was exposed to NMP during gestation[8]. These findings suggest potential health hazards in humans.

Excluding one report of an experimental inhalation study in humans[9], no pharmacokinetic study has been done. Traditionally, observation-based approaches in various fields have been used to establish dose (exposure)-response (concentrations in biological specimens) relationships. Pharmacokinetic modeling is an alternative model-oriented approach, which enables us to obtain dose-response relationships from a small number of experiments. It is a useful approach for predicting the kinetics of chemicals under various conditions. The aim of this study is to construct a simple and informative pharmacokinetic model of NMP for biological monitoring.

Materials and Methods

Pharmacokinetic modeling

We developed a pharmacokinetic model for illustrating the metabolic fate of NMP in plasma and urine. This model is composed of one compartment (Fig. 1). The differential equations describing the metabolic fates of NMP were numerically solved by software named ITHINK (Systems inc. Hanover, NH, USA). In the simulation, we assumed that the concentrations of NMP in urine adjusted by creatinine concentrations are proportional to the concentrations of NMP in plasma. The coefficient K in Fig. 1 was estimated by using an observed statistical regression analysis of plasma and urinary concentrations for each compound. Parameters were optimized by a nonlinear least squares method as previously reported[10, 11].

Factory A

Factory A manufactured lenses for optical instruments. The glass pieces (4 cm in diameter × 1 cm of thick) were polished several times and coated with a thin film of covering material. After coating, the lenses were washed with a solvent to remove the oil layer on the surface. The lenses were washed in a room with a large washing chamber (1 m × 1 m × 2.5 m). Four workers were engaged in the washing process. Tetrachloroethylene had been used as a washing solvent before 1997 and has been replaced by NMP. The lenses (3 cm in diameter) are washed in the same washing chambers as were used for tetracholoroethyelene without any modification. Fifty lenses were put in a special container basket. Workers opened a door of the chamber to dip the basket into a pool containing NMP inside the chamber. After closing the door, sonication was started to wash the lens surfaces. This washing process lasted for 5 min. After washing, the baskets were lifted from the pool and then dried outside the chamber for a couple of minutes. Although drops of NMP were removed from the chamber as thoroughly as possible, NMP adhering to the surface of lenses and baskets evaporated in the work room air. After drying for a couple of minutes, the baskets were then conveyed to the next washing step in the closed chamber.

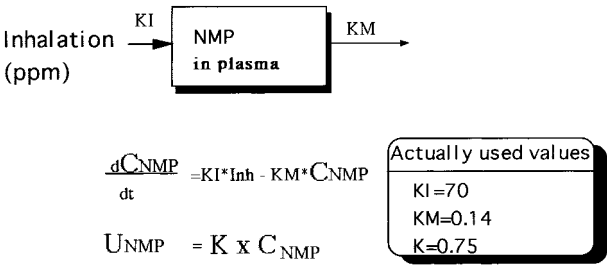

Fig. 1. A one compartment pharmacokinetic model.

Four workers enrolled in this study and were followed up for a full week. They started working at 8:00 am and finished at 8:00 pm with a one-hour lunch break at noon from Monday to Friday. On Monday, the first day of the work week, we collected blood and urine samples at the start of work (8:00 am), at noon (12:00), and at 5:00 pm. At 8:00 pm only urine samples were collected. From Tuesday to Thursday urine samples were collected at 8:00 am and 8:00 pm. On Friday, urine samples were collected at 8:00 am and 5:00 pm. Blood samples were also collected at 5:00 pm.

Five volunteers stayed in the workroom on Monday and observed the washing process from 8:00 am to 5:00 pm. Blood and urine samples were collected at 8:00 am, at noon, at 5:00 pm, and 8:00 pm on Monday, at 8:00 am and 5:00 pm on Tuesday and at 8:00 am on Wednesday.

Factory B

The major products of factory B were parts of wire apparatuses. Workers washed metal parts (1 cm in size) in NMP in a work room. They put more than 100 parts in a basket, opened the cover of a tank containing NMP and dumped the basket into the tank. The lid of the container was then closed. After 30 min of soaking in NMP, the basket was lifted out and immediately transferred to a tank containing water. Some drops adhering to the parts and baskets were scattered over the floor. Other drops were carried into the water tank. NMP evaporated into the washing room air from the contaminated floor and water tank. Eight workers were engaged in this process, all of whom were enrolled in this study. Their work week was from Monday to Friday, 8:00 am to 8:00 pm with a one-hour lunch break at noon. Blood and urine samples were collected on Monday at the beginning and end of work.

Analytical method for NMP

Blood samples were collected in evacuated heparinized

tubes. Blood samples were centrifuged at 1,500 g for 10 min and the plasma was frozen and kept at −80°C until analysis. Urine was collected in polyethylene bottles, frozen, and stored at −80°C. Plasma or urine (1 m*l*) was transferred into a glass tube, to which toluene (1 m*l*) and 12 M potassium hydroxide (2 m*l*) containing 0.25% ammonia were added. After shaking for 10 min, the toluene phase was transferred to vials.

The adsorbent (1 μl) was directly injected into a GC-MS (G1800 GCD system, Hewlett Packard Co., Wilmington, DE, U.S.A.) equipped with an automatic sample injector (HP 6890 Series Injector, Hewlett Packard Co.). An HP-5MS capillary column (30 m length, 0.25 mm in diameter, 0.25 μm in film thickness, supplied by Hewlett Packard Co., U.S.A. Wilmington, DE, U.S.A.) was used. The injection port and transfer lines were heated to 320°C. The carrier gas was helium and the flow-rate was 1 m*l*/min with splitless nodes. A thermal control program kept the oven temperature at 40°C for 1 min, then increased it at the rate of 30°C/min to 250°C. The concentration of NMP was determined from the signals of selected ion fragments at 99 m/z in dichloromethane by SIM (Selected Ion Method). The detection limit was 10 ng/m*l*.

Personal exposure monitoring

The NMP levels were measured with a diffusive sampler in which activated charcoal was used as an absorbent (Passive Gas Tube, Shibata Scientific Technology, Ltd). The activated charcoal absorbent was eluted with 2 m*l* dichloromethane, and the diffusive sampler was eluted with 1.5 m*l* dichloromethane. The adsorbent (1 μl) was directly injected into a GC-MS (G1800B GCD system, Hewlett Packard Co., Wilmington, DE, USA). A capillary column HP-5MS (30 m length, 0.25 mm in diameter, 0.25 μm in film thickness, supplied by Hewlett Packard Co., U.S.A. Wilmington, DE, U.S.A.) was used. Injection and transfer lines were heated to 320°C. The carrier gas was helium, and the flow-rate was 1 m*l*/min. The thermal control program kept the oven temperature at 40°C for 1 min, then increased it at the rate of 30°C/min to 250°C. The concentration of NMP was determined from the signals of selected ion fragments at 99 m/z in the dichloromethane by a selected ion method. The detection limit was 0.01 ppm for 8 h.

Participants

The means and standard deviations (range) of age, height (cm), body weight (kg) and body mass index were; 25.2 ± 14.7 (20–44), 174 ± 7.0 (165–183), 67.6 ± 11.6 (52.0–85.0) and 22.6 ± 4.3 (19.3–29.8) for five workers in factory A; 39.8 ± 5.9 (29–45), 169 ± 5.2 (160–175), 67.8 ± 13.9 (50–85) and 23.6 ± 4.0 (18.8–28.4) for five volunteers; 49.8 ± 4.21 (45–56), 162 ± 5.7 (154–170), 58.4 ± 4.8 (53.5–65) and 22.5 ± 1.2 (20.6–24.1) for eight workers in the factory B. These workers and volunteers had taken annual medical checkups including RBC, WBC, Hb, AST, ALT, r-GTP, total cholesterol, HDL cholesterol, triglyceride, ECG and plain chest roentgenogram. We obtained informed consents from all participants in this study.

During this study, no restrictions on diet, water consumption or beverages were requested. Workers and volunteers followed their usual eating and drinking habits.

Statistical analysis

All statistical analyses were conducted with SAS packages (SAS Institute Inc). A p value of <0.05 was considered significant throughout this study.

Results

In both factories A and B, workers were exposed to NMP for 12 h daily. Weekly time-weighted averages for individual workers were less than 0.5 ppm, ranging from 0.04 to 0.69 ppm. Volunteers who observed the workers in the factories were exposed to NMP at comparable dose levels of NMP (Table 1). Workers were protected with gloves and aprons from direct contact with NMP drops.

Table 1. Exposure levels in workers and volunteers

Factory			Time-weighted average		Exposed
			Weekly mean ± SD[a]	Range[b]	hours
A	Worker	a	0.42 ± 0.14	0.69–0.28	12
		b	0.24 ± 0.09	0.40–0.14	
		c	0.14 ± 0.05	0.21–0.09	
		d	0.19 ± 0.06	0.28–0.24	
	Volunteers	n=5	0.28 ± 0.03	0.32–0.24	8
B	Workers	n=8	0.33 ± 0.20	0.59–0.04	12

In the factory A, four workers (a to d) were followed up for an entire workweek. Volunteers and workers in the factory B were observed for only a single day. [a] Means and SD of TWAs. [b] The range of TWAs.

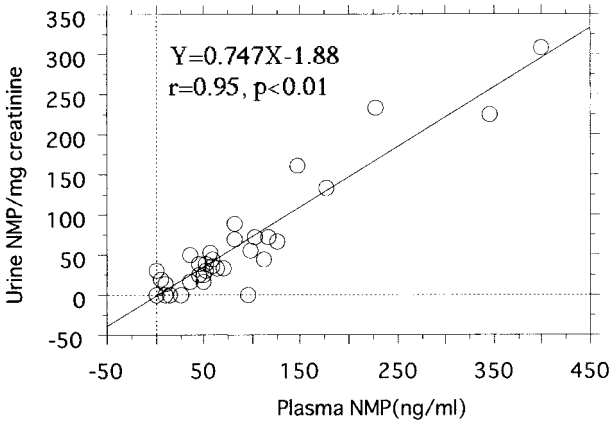

Fig. 2. Relationships between concentrations of NMP in plasma (X) and urine standardized by creatinine (Y) in four workers (a to d) and five volunteers (a to e). The total number of samples analyzed was 51.

Our observation at the workplace suggests that inhalation is the most likely route of exposure to NMP. Medical records demonstrated that none of the participants had abnormal values in those checked items. One of the workers had dermatitis after a direct contact with NMP on the skin.

In factory A, blood and urine samples revealed that the NMP concentration in plasma (X) was correlated with its concentration in urine (Y), represented by a simple equation as shown in Fig. 2. By multiplying plasma concentration by 0.75, we simulated the concentration in urine in the pharmacokinetic model.

We developed a pharmacokinetic model composed of one compartment to illustrate the kinetics of NMP. We simulated concentrations of NMP in plasma and urine for individual workers (a to d) and volunteers (a to e) (Fig. 3). Fig. 4 summarizes the relationships between

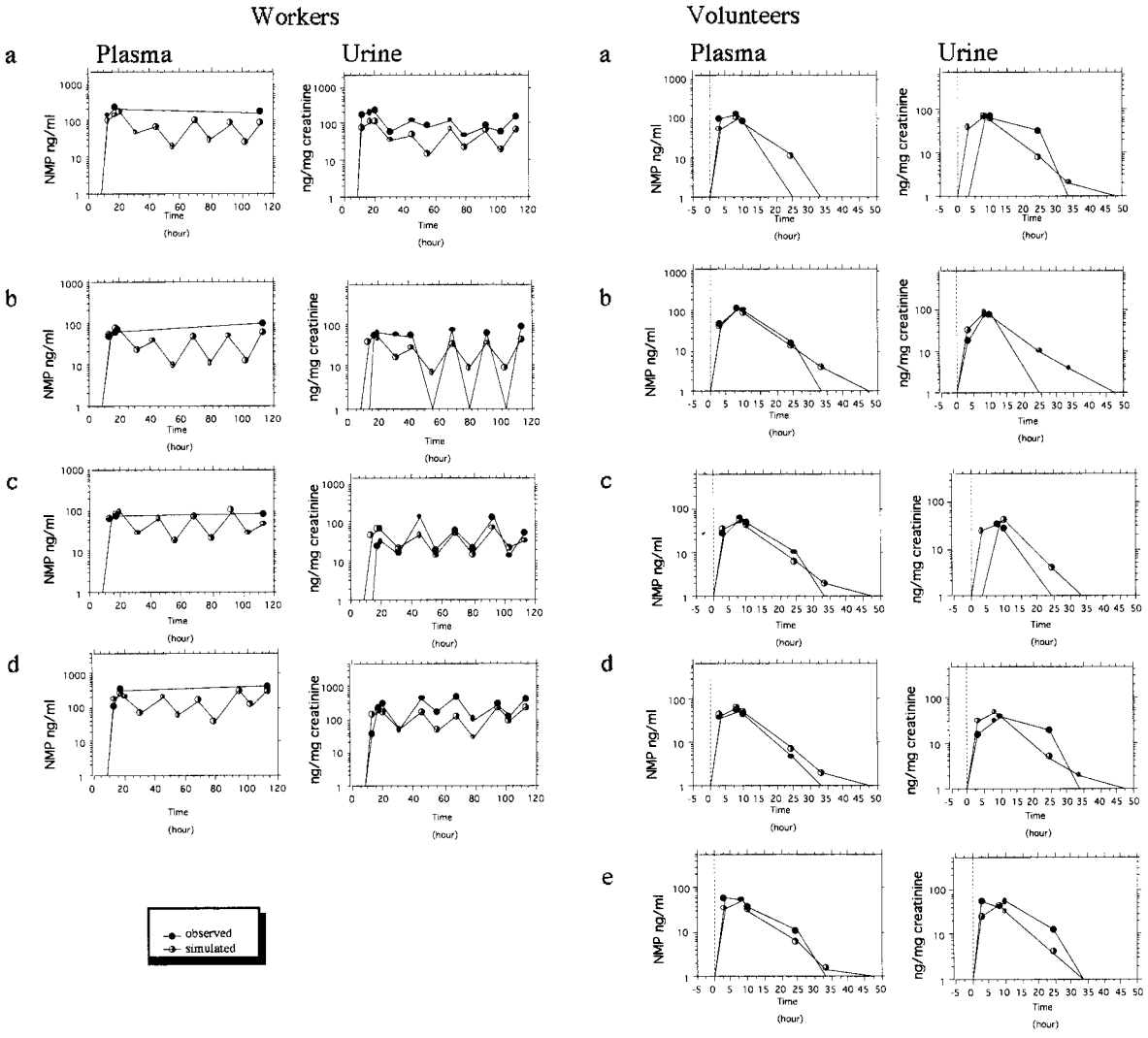

Fig. 3. Simulated and observed values in individual workers (a to d) in factory A and volunteers (a to e).

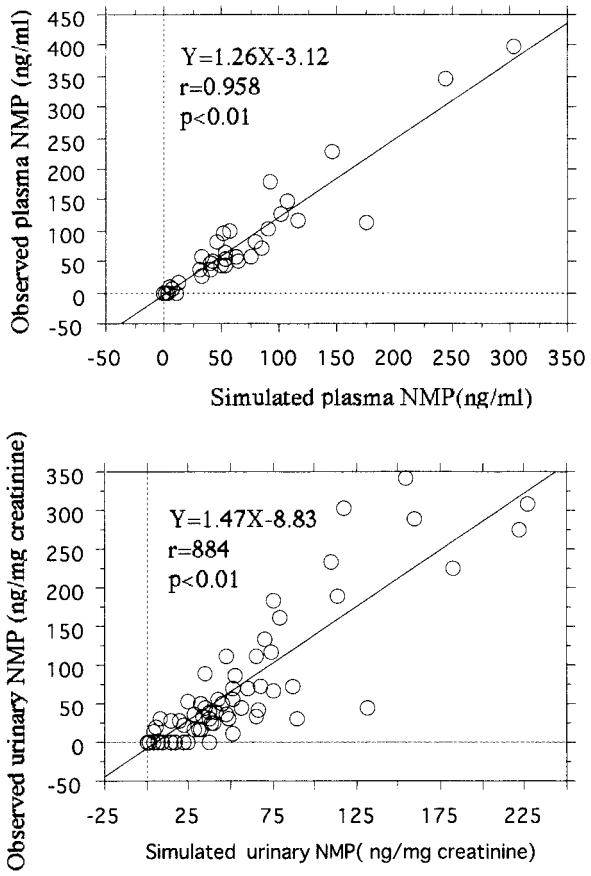

Fig. 4. Relationships between observed (Y) and simulated values (X) in workers in factory A and volunteers. The numbers of samples were 51 for plasma and 83 for urine.

the observed NMP values in plasma and urine and the predicted NMP values in plasma and urine.

To investigate the predictability of pharmacokinetic modeling, we then applied this model to workers in factory B. We collected plasma and urine samples before work and at the end of work. The concentrations of NMP in both plasma and urine samples before work were below the detection limit. Concentrations of NMP observed in plasma (Yp) and urine (Yu) at the end of work showed strong agreement with the corresponding predicted values (Xp and Xu): Yp=1.131Xp-0.938, r=0.974, p<0.0001, n=8; Yu=1.591Xu-23.9 r=0.909, p<0.0001, n=8).

Experimental exposure of human male volunteers to NMP has been reported[9]. The exposure levels were 10 mg/m^3 (2.5 ppm), 25 mg/m^3 (6.2 ppm) and 50 mg/m^3 (12.4 ppm) for 8 h. In the report, concentrations of NMP in plasma and urine were reported. In the present study, we simulated the pharmacokinetics of NMP in plasma and urine during and after 8-h exposures to 2.5 ppm, 6.2 ppm and 12.4 ppm to test the predictability of the model simulation (Fig. 5). Irrespective of slight differences - standardized by the creatinine concentration in our study but not in the reported study - urine values show good agreement. The plasma values were also well simulated by the model.

Finally, the developed model was applied to biological monitoring of NMP exposure. The plasma and urinary concentrations of NMP were simulated for certain conditions - 8 h of exposure from Monday to Friday at 0, 0.5, 1.5, 4.5 and 13.5 ppm without fluctuation. Simulations showed that concentrations of NMP in plasma and urine before work were increased from Monday to Friday (data not shown). The values at the end of 8-h exposure also increased from Monday to Friday by 3%, indicating that concentrations of NMP in urine and plasma at the end of work can be used as exposure biomarkers with a negligible effect of accumulation of NMP, if any. To accommodate uncontrollable variations including individual variations, we presented a range as the lowest and highest values, which correspond to two-fold smaller or two-fold greater values than the actual value (Fig. 6). The reported values[9] fall within these ranges.

Discussion

We reported here a simple and informative pharmacokinetic model for illustrating the kinetics of NMP in humans. With this model, we successfully predicted the concentrations of NMP in plasma and urine under various conditions by simply changing the exposure parameters.

NMP is reported to be readily eliminated from the body with a short half-life in plasma[9, 12]. The elimination is mainly by metabolism to other compounds in the body. The present pharmacokinetic model can successfully simulate plasma and urine concentrations of NMP after exposure at doses from less than 1 ppm up to 12 ppm. This universality indicates that a linear pharmacokinetic model can safely be applied to exposures at about 10 ppm without metabolic saturation.

In both developed and developing countries, industries are under increasing pressure to replace chlorinated hydrocarbons with alternate chemicals that are less toxic to humans and less hazardous to the environment. The absence of ozone depleting activity and relatively low organ toxicity lends NMP to its current use in many industries, leading to a dramatic increase in its production in recent years. From a toxicology viewpoint, it is readily absorbed by the skin and is suspected to have developmental toxicity, so that a rational occupational protection strategy would be to monitor internal dose levels of NMP by biological monitoring. NMP levels in plasma and urine can be used as biomarkers for biological monitoring as shown in the present study.

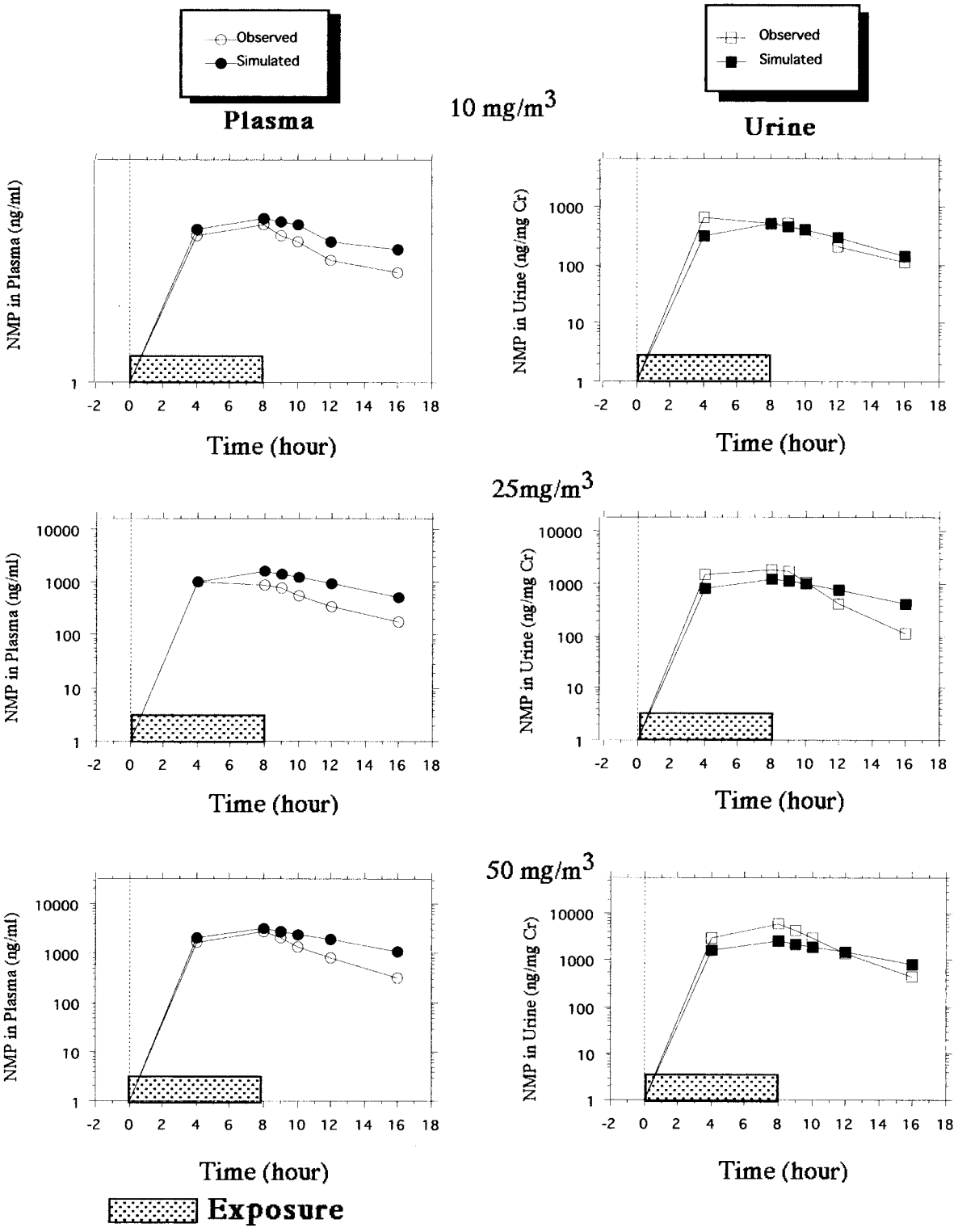

Fig. 5. Prediction of kinetics of NMP in plasma and urine at 10 (2.5 ppm), 25 (6.2 ppm) and 50 (12.4 ppm) mg/m^3 for eight hours in humans. Observed values are the values in the literature[9], whereas simulated values indicate values obtained by simulation. Reported values in urine (mg/*l*) were converted to the values (ng/mg of creatinine) for comparison on the assumption that one liter of urine contains 1,000 mg of creatinine.

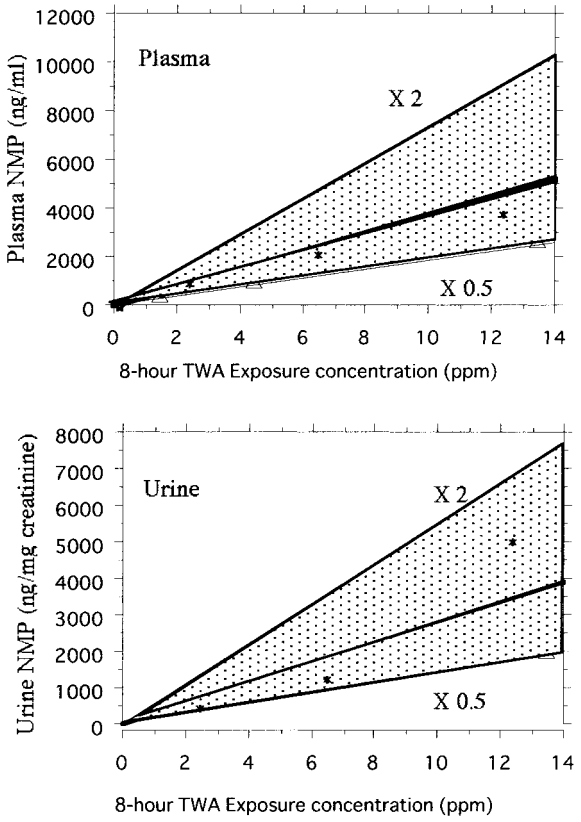

Fig. 6. Predicted concentrations of NMP in plasma and urine at the end of 8 h of work after exposure at various TWA levels. The simulation condition includes 8-h exposure with a one-hour lunch break. The values are those at the end of exposure on the first day of the work week. (*) indicates reported values[9].

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