0090-9556/03/3105-659–669$7.00
DRUG METABOLISM AND DISPOSITION
Vol. 31, No. 5
994/1062080
DMD 31:659–669, 2003
Printed in U.S.A.
Copyright © 2003 by The American Society for Pharmacology and Experimental Therapeutics

DRUG METABOLISM & DISPOSITION

TOXICOKINETICS AND METABOLISM OF *N*-[^{14}C]*N*-METHYL-2-PYRROLIDONE IN MALE SPRAGUE-DAWLEY RATS: IN VIVO AND IN VITRO PERCUTANEOUS ABSORPTION

JEAN-PAUL PAYAN, ISABELLE BOUDRY, DOMINIQUE BEYDON, JEAN-PAUL FABRY, MARIE-CHRISTINE GRANDCLAUDE, ELISABETH FERRARI, AND JEAN-CLAUDE ANDRÉ

Institut National de Recherche et de Sécurité (I.N.R.S.), Vandoeuvre, France

(Received November 13, 2002; accepted February 13, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Neat *N*-methyl-2-pyrrolidone (NMP) rapidly penetrated into the skin of male Sprague-Dawley rats after in vivo and in vitro topical application. At the two topical doses tested in vivo, no steady state was observed. The maximal absorption fluxes were 10 and 20 mg/cm^2/h for 20 μl/cm^2 and 40 μl/cm^2, respectively. Similar results were observed after in vitro topical application of neat [^{14}C]NMP (25–400 μl/cm^2) in fresh full-thickness skin. Whatever the dose tested, the percutaneous absorption fluxes increased with exposure time to reach a maximum value (F_{max}) and then decreased. F_{max} and the time to reach it (T_{max}) increased as the dose increased. At the highest dose, which may be considered as an "infinite dose," the maximal flux (7.7 ± 1.1 mg/cm^2/h, n = 12) occurred 6 h after the topical application of NMP. The decrease on percutaneous absorption flux was correlated with the dilution of neat NMP with water from the receptor fluid. A semi-quantitative mathematical model was developed to describe the absorption flux of NMP taking into account the transfer of water through the skin. The K_p values determined from the different aqueous solutions of NMP (1:1 to 1:32, v/v) were not significantly different. The mean value was 6.4 (10^{-3} cm/h) (range, 4.7 to 7.6). Occlusion did not affect the percutaneous absorption flux of neat NMP. Desquamation increased the percutaneous absorption of NMP slightly. The skin did not metabolize NMP. The flux was dependent on the thickness of the skin and was proportional to the concentration of NMP. These findings suggest a passive diffusion of NMP through the skin.

N-Methyl-2-pyrrolidone (NMP) is a limpid liquid, which is soluble in water and a wide range of organic solvents. Its volatility is low (vapor pressure 32 Pa at 25°C). The use of NMP as a solvent is increasing, in particular as a substitute for methylene chloride in paint strippers. One of the major uses of NMP is the extraction of aromatics from lubricating oils. NMP is also used as a vehicle for drugs or to facilitate their percutaneous absorption.

Acute toxicity is low. LD_{50} in rabbits was 4 to 8 g/kg and 1.5 to 7 g/kg in rats after topical application. The LD_{50} in mice after intravenous (i.v.), intraperitoneal, and oral administration was 3.5, 4.3, and 7.5 ml/kg, respectively. In rats, the LD_{50} was 2.2, 2.4, and 3.8 ml/kg, respectively (Bartsh et al., 1976). Studies on reproductive toxicity have shown that NMP causes developmental toxicity at doses causing no or mild maternal toxicity (Hass et al., 1994; Solomon et al., 1995). Cases of stillbirth after occupational exposure to NMP have been reported (Solomon et al., 1996; Bower, 1997).

NMP is well absorbed through gastrointestinal, pulmonary tract, and skin (Midgley et al., 1992; Akesson and Paulsson, 1997; Ursin et al., 1999). Metabolites of NMP are intensively excreted in urine mainly as 5-hydroxypyrrolidone (5-HNMP) (Wells et al., 1992; Akesson and Jonsson, 1997; Payan et al., 2002).

A percutaneous absorption rate of 25.3 μg/cm^2/h for NMP has been calculated from an in vivo experiment in rats with a co-exposition of NMP and 2-vinylpyrrolidone (Midgley et al., 1992). This value is about 3 orders of magnitudes lower than the flux determined (17 mg/cm^2/h) in human skin (Ursin et al., 1999). The low percutaneous flux determined in rats is incompatible with the use of NMP as a percutaneous absorption enhancer for drugs (Barry and Bennett, 1987; Sugibayashi et al., 1989).

Thus, this work was carried out to determine in vivo and in vitro the percutaneous absorption of neat [^{14}C]NMP in rats. Additional experiments were also conducted in vitro with aqueous solutions of NMP (1:2–1:32, v/v).

Materials and Methods

Chemicals. Radiolabeled *N*-[^{14}C]methyl-2-pyrrolidone ([^{14}C]NMP) was supplied by Amersham International Plc (Buckinghamshire, England). Radiochemical purity exceeding 99% was determined by HPLC before each experiment. The specific activity was 1.04 GBq/mmol (28 mCi/mmol).

Animals. Male haired Sprague-Dawley rats (Iffa Credo, Saint-Germain-sur-l'Arbresle, France) weighing 250 to 300 g were used for all studies. The animals were acclimatized to laboratory conditions for at least 4 days prior to initiating the studies in rooms with a 12-h light/dark cycle, that were designed to control relative humidity at 50 ± 5% and temperature at 22 ± 1°C. Commercial food pellets (UAR Alimentation-Villemoison, Epinay-sur-Orge, France) and tap water were available ad libitum.

This study was sponsored by Public Health Service Grant NCCAM R21 AT00511-01.

[1] Abbreviations used are: NMP, *N*-methyl-2-pyrrolidone; LD_{50}; 5-HNMP, 5-hydroxy-NMP; HPLC, high performance liquid chromatography; ANOVA, analysis of variance; F_{max}, maximal percutaneous absorption flux; F_{max} Nor, F_{max} for a skin thickness of 1.3 mm; AUC, area under the plasma curves.

Address correspondence to: Dr. Jean Paul Payan, Institut National de Recherche et de Sécurité, Avenue de Bourgogne, B.P. No. 27, 54501 Vandoeuvre Cedex, France. E-mail: payanjp@inrs.fr

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

In Vivo Percutaneous Penetration and Absorption of [^{14}C]NMP by Sacrifice. One day before dosing, the middle of the back of the rats was clipped with electric clippers, and a circular ring (10 cm^2) was glued. After topical application of neat [^{14}C]NMP, 20 or 40 μl/cm^2, the skin was covered by a perforated circular plastic cap to allow aeration. Batches of three to five hairy male rats were sacrificed at different times (0.5, 0.75, 1, and 2 h) after dosing by bleeding the abdominal aorta under mild ether anesthesia. The blood was collected on heparin. After sacrifice, the skin area of the application site was washed five times with 200 μl of water to remove the unabsorbed fraction of NMP. A preliminary study showed that 5 min after topical application of [^{14}C]NMP, more than 95% of the radioactivity was removed by this washing process ($n = 3$). The radioactivity of the application skin area and the skin area around the ring (about 30 cm^2) was measured after digestion in KOH solution. Radioactivity in the carcass and excreta (urine and feces) was also analyzed.

In Vivo Percutaneous Penetration and Absorption of [^{14}C]NMP by Catheterism. For the sequential collection of urine and blood, a catheter was introduced into the carotid artery and bladder, respectively, 1 week before topical administration of [^{14}C]NMP. The catheters (internal diameter, 0.58 mm; external diameter, 0.96 mm) were introduced subcutaneously, exteriorized through the back of the neck, and inserted into a protective stainless tubing (about 2 g in weight) ligatured firmly to the skin. Urine was excreted by injecting saline solution (2 ml) into the bladder. Blood was collected on heparin. The rats were clipped 1 day before dosing. Neat [^{14}C]NMP (20 μl/cm^2) was applied on the skin (10 cm^2). The application area was washed with water 24 h after dosing. Blood and urine were collected at different times until the animal was sacrificed (72 h).

In Vitro Percutaneous Absorption of NMP. In vitro percutaneous absorption was assessed with static diffusion cells using fresh full-thickness skin of male rats (0.9–1.5 mm, 1.3 ± 0.0, $n = 73$). The rats were sacrificed with Pentobarbital. The whole dorsal region was shaved, and the excess of subcutaneous tissue carefully removed. The skin section was cut into circular sections (four per rat, 1.76 cm^2) and placed, stratum corneum side up, in diffusion cells. The diffusion cells were maintained at a temperature of 36°C with a circulating water bath, yielding a skin surface temperature of 32 ± 1°C. The dermis side was kept in contact with the RPMI receptor fluid (Life Technologies, Paisley, Scotland) containing 2% bovine albumin and 1% penicillin-streptomycin. The fluid receptor was previously filtered through a sterile Millex (Millipore, Bedford, MA) 0.22-μm pore size filter and degassed with a vacuum pump. Preliminary experiments had shown that absorption flux was not significantly different when the receptor fluid was NaCl 0.9%. The integrity of the skin samples was assessed by determining the *trans*-epidermal water loss (Tewameter, TM210, Courage + Khazaka) after an equilibrium time of 1 h.

Neat or aqueous solutions of [^{14}C]NMP were applied on a skin surface area of 1.76 cm^2. The cells were non-occluded. An aliquot (200 μl) of receptor fluid (5.15 ml) was collected at regular intervals from 24 to 52 h after sacrifice with an automatic fraction collector (Gilson FC 204, Middleton, WI). The same volume of fresh receptor fluid was automatically introduced into the cell to maintain the volume of the receptor fluid constant. At the end of the experiment, the unabsorbed dose of [^{14}C]NMP was removed with water (1 × 500 μl) and cotton swabs. The skin was digested in KOH (25%, w/v). The radioactivity contained in the receptor fluid samples, the washing water, and the skin homogenates was measured by adding 10 ml of liquid scintillation solution (Pico Fluor 30; Packard, St. Louis, MO). Counting efficiency was determined by quenching correction curves for the various addition and scintillation fluids with a liquid scintillation spectrophotometer (CA 1900; Packard).

Metabolism of NMP. Neat NMP was applied (200 μl/cm^2) on fresh skin samples of one rat. After 2 h of exposure, the radioactivity contained in the receptor fluid, and skin homogenates in water were analyzed by the HPLC method described below.

Reproducibility. Maximal percutaneous absorption rate (F_{max}) and the time to reach it (T_{max}) were determined in three independent experiments (three rats, two skin samples per rat) with neat NMP (400 μl/cm^2) for 24 h of exposure. F_{max} was normalized for a skin thickness of 1.3 mm according to eq. 1.

$$F_{max}\ \text{Nor} = F_{max} \times \text{thickness of the skin sample}/1.3$$

Effect of Neat NMP Doses. F_{max} Nor and T_{max} were determined after topical application of neat NMP (25–400 μl/cm^2) for a 24-h exposure period.

Effect of Hydration on NMP Absorption Flux. Different doses of neat [^{14}C]NMP (100, 200, and 400 μl/cm^2) or ^3H_2O (400 μl/cm^2) were applied on skin samples, and aliquots of the receptor fluid were collected for 4 h. At the end of the experiment, the volumic radioactive concentration of unabsorbed NMP was determined. In a second experiment, neat [^{14}C]NMP was applied on the skin of male rats (400 μl/cm^2) aliquot of unabsorbed dose (10 μl) and of the receptor fluid (160 μl) were collected at different times for 30 h to determine the evolution of the volumic radioactive concentration of the unabsorbed dose and percutaneous absorption fluxes, respectively. Additionally, neat NMP (400 μl/cm^2) was introduced on a glass watch, and aliquots of NMP were collected to measure the radioactive concentration.

Effect of NMP on the Transfer of ^3H_2O from the Receptor Fluid. Four groups of excised skin ($n = 3$) were treated as follows.

Group 1: 400 μl of water were deposited on skin sample and ^3H_2O was introduced into the receptor fluid to measure the transfer of water from receptor fluid to the deposited water on skin.

Group 2: 400 μl of ^3H_2O were deposited on skin to measure the transfer of water from the deposited area to the receptor fluid.

Group 3: 400 μL of NMP were deposited on skin and ^3H_2O was introduced into receptor fluid to measure the transfer of water from the receptor fluid to deposited skin NMP.

Group 4: 400 μl of [^{14}C]NMP were deposited on skin to measure the absorption flux of NMP.

At the end of the exposure (4 h), unabsorbed water or NMP was collected to measure ^3H_2O content for groups 1 and 3. For groups 2 and 4, radioactivity present in the receptor fluid was analyzed.

Effect of Aqueous Dilution of NMP. An identical volume of aqueous dilutions of NMP (400 μl/cm^2) at different concentrations (1:2 to 1:32, v/v) was applied on the skin. Percutaneous absorption was determined for 24 or 52 h.

Effect of Occlusion or Desquamation. Percutaneous absorption fluxes of neat NMP (400 μl/cm^2) were compared between skin samples of haired rats, with or without occlusion. Percutaneous absorption fluxes of neat NMP (100 μl/cm^2) were compared between skin samples of hairless rats after desquamation (20 stripping, 100 g/cm^2) or without desquamation.

HPLC Analysis. An HPLC method to analyze unchanged NMP and its main metabolites has been previously described (Payan et al., 2002). Briefly, proteins from plasma were precipitated in methanol. Unchanged NMP and its main metabolites (5-HNMP, 2-hydroxy-*N*-methylsuccinimide, and *N*-methylsuccinimide) contained in methanolic phase from plasma or aliquot of urine are analyzed by HPLC with a mixture of H_2SO_4 0.001 N and acetonitrile (85:15, v/v). The radioactivity contained in the HPLC eluates was measured with a liquid scintillation spectrophotometer.

Analysis of Radioactivity. Samples of urine (500–1000 μl) and plasma (500 μl) were accurately weighed and added directly to liquid scintillation vials containing 10 ml of liquid scintillation solution (Pico Fluor 30; Packard). Samples of fresh feces were weighed and homogenized in water (1:5, w/v) in glass vials. Tissues (liver or kidney) were homogenized in water (1:5, w/v). Aliquots of feces or tissue homogenates (250–500 mg) were mixed with 10 ml of Pico Fluor 30. The radioactivity of all the samples was measured in a Packard liquid scintillation spectrophotometer model 1900. Counting efficiency was determined by quenching the correction curves of the various additions and scintillation fluids.

Expression of Data and Statistical Analysis. Values were expressed as the percentage of [^{14}C]NMP dose per organ (%Q_o/organ) or as the percentage of [^{14}C]NMP dose per gram (%Q_o/g) of fresh tissue. The one-way ANOVA test was used to determine the significance of the means. The level of significance was set at $p < 0.05$.

The terminal elimination rate (β) of NMP and its main metabolites in plasma were obtained by log-linear concentration time data. The area under the plasma curves of NMP and its metabolites from time 0 to the end of the experiment ($AUC_{0\text{-}t}$) were calculated by the linear trapezoidal rule. The AUC from infinity was estimated by the calculated concentration at t divided by β. The sum of both areas was $AUC_{0\text{-inf}}$.

The percentage of the absorbed dose was calculated from:

a) the radioactivity content in the excreta and carcass

$$\%\ \text{absorbed dose} = \%\ \text{applied dose in urine} + \text{feces} + \text{carcass} \quad (1)$$

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

TABLE 1

Mass balance of ^{14}C after a topical application of neat [^{14}C]NMP in male Sprague-Dawley rats

Batches of rats were sacrificed at different times after a topical application of neat [^{14}C]NMP (20 μl/cm^2, 10 cm^2). Values are expressed as mean ± S.E.M. (n = 4).

	15 min	30 min	45 min	60 min	120 min
Urine[a]	0.01 ± 0.00	0.03 ± 0.04	0.14 ± 0.03	0.19 ± 0.13	1.07 ± 0.37
Carcass and Tissues[a]	5.5 ± 0.18	15.90 ± 2.47	25.03 ± 2.45	34.18 ± 3.06	55.79 ± 1.58
Collected blood[a]	0.4 ± 0.01	1.89 ± 0.28	1.35 ± 0.11	1.78 ± 0.11	2.89 ± 0.05
Washing cage[a]	0.1 ± 0.05	0.21 ± 0.08	0.21 ± 0.04	0.29 ± 0.06	0.48 ± 0.22
Application site skin[a]	10.4 ± 0.69	15.23 ± 0.83	12.80 ± 1.03	14.42 ± 0.94	15.84 ± 1.48
Skin around the application site skin[a]	2.7 ± 2.55	1.94 ± 2.50	0.90 ± 0.07	2.17 ± 4.58	1.78 ± 0.27
Washing of the application site skin[a]	77.8 ± 3.14	61.20 ± 2.78	51.08 ± 3.42	34.77 ± 7.98	9.83 ± 1.91
Recovery[a]	96.8 ± 0.54	97.46 ± 3.59	93.43 ± 1.33	93.79 ± 1.72	91.22 ± 1.13
Percentage of the absorbed dose	6.0 ± 0.19	18.03 ± 2.75	28.65 ± 2.77	36.44 ± 3.33	60.23 ± 1.66
Percentage of the penetrated dose	18.4 ± 3.11	35.20 ± 1.45	42.34 ± 2.13	52.67 ± 7.29	77.85 ± 0.99
Penetration flux (μl/cm^2/h)	4.8 ± 0.15	7.21 ± 1.10	7.64 ± 0.74	7.29 ± 0.67	6.02 ± 0.17
Absorption flux (μl/cm^2/h)	14.7 ± 2.49	14.08 ± 0.58	11.29 ± 0.57	10.53 ± 1.46	7.79 ± 0.10
Plasma concentration (percentage of the dose/g)	0.045 ± 0.001	0.096 ± 0.015	0.17 ± 0.02	0.21 ± 0.02	0.35 ± 0.03
Application site skin (μl/cm^2)	2.08 ± 0.14	3.05 ± 0.17	2.56 ± 0.21	2.88 ± 0.19	3.17 ± 0.30
Skin around application site skin (μl/cm^2)	0.54 ± 0.51	0.39 ± 0.50	0.18 ± 0.01	0.43 ± 0.92	0.36 ± 0.05
Skin (g/cm^2)	0.30 ± 0.02	0.37 ± 0.01	0.27 ± 0.01	0.27 ± 0.00	0.28 ± 0.02
Liver/plasma	0.69 ± 0.01	0.71 ± 0.01	0.72 ± 0.00	0.73 ± 0.00	0.74 ± 0.01
Kidney/plasma	0.86 ± 0.03	0.83 ± 0.03	0.88 ± 0.09	0.84 ± 0.01	0.85 ± 0.01
NMP	0.04 ± 0.00	0.09 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	0.31 ± 0.02
5-HNMP	0.000 ± 0.000	0.001 ± 0.002	0.005 ± 0.002	0.008 ± 0.002	0.024 ± 0.004

[a] Percentage of the dose.

b) the ratio of the AUC of NMP or 5-HNMP after topical application versus intravenous administration (500 mg/kg)

$$\%\ \text{absorbed dose} = \frac{\text{AUC}_{\text{0-inf topical application (\% dose/ml}\times\text{h}\times 100)}}{\text{AUC}_{\text{0-inf (\% dose/ml}\times\text{h)}}} \quad (2)$$

c) the ratio of the total radioactivity, NMP, or 5-HNMP excreted in the urine after topical application versus intravenous administration (500 mg/kg)

$$\%\ \text{absorbed dose} = \frac{\%\ \text{topical dose in urine} \times 100}{\%\ \text{intravenous dose in urine}} \quad (3)$$

Intravenous values are from Payan et al. (2002).

Results

Percutaneous Absorption of NMP. The in vivo percutaneous absorption of [^{14}C]NMP was determined by sacrificing the animals at different times after topical application of neat [^{14}C]NMP (20 and 40 μl/cm^2). After topical application of 20 μl/cm^2 of neat NMP, the percentage of the absorbed dose increased rapidly with exposure time and accounted for about 60% of the dose after 2 h of exposure (Table 1). The absorption flux was maximal after 30 min of exposure (9.7 mg/cm^2/h) and then decreased (Fig. 1). A similar result was obtained with a 40 μl/cm^2 dose, the maximal percutaneous absorption flux being 23.4 ± 3.4 mg/cm^2/h after 45 min of exposure.

The time course of [^{14}C]NMP, unchanged NMP, and 5-HNMP in plasma and urinary excretion after topical application of 20 μl/cm^2 are presented in Figs. 2 and 3. The unchanged NMP plasma levels were maximal 4 h after the beginning of exposure (0.29 ± 0.01 percent of the applied dose; 0.58 mg/ml) and represented more than 90% of the total radioactivity in the plasma. The 5-HNMP plasma level was more or less constant between 6 and 24 h (0.039% of the applied dose per milliliter, 0.08 mg/ml). The NMP urinary excretion rate is peaked between 3 and 4 h after exposure (0.65 ± 0.1% of the dose; 1.3 mg/h). The total urinary excretion of NMP was 6.9 ± 0.3 percent of the applied dose. The urinary excretion rate of 5-HNMP reached a peak between 9 and 24 h (1.7% of the applied dose/h, 3.4 mg/h).

For a 24-h dermal exposure to neat [^{14}C]NMP (20 μl/cm^2), the percentage of the absorbed dose calculated from the content of radio-

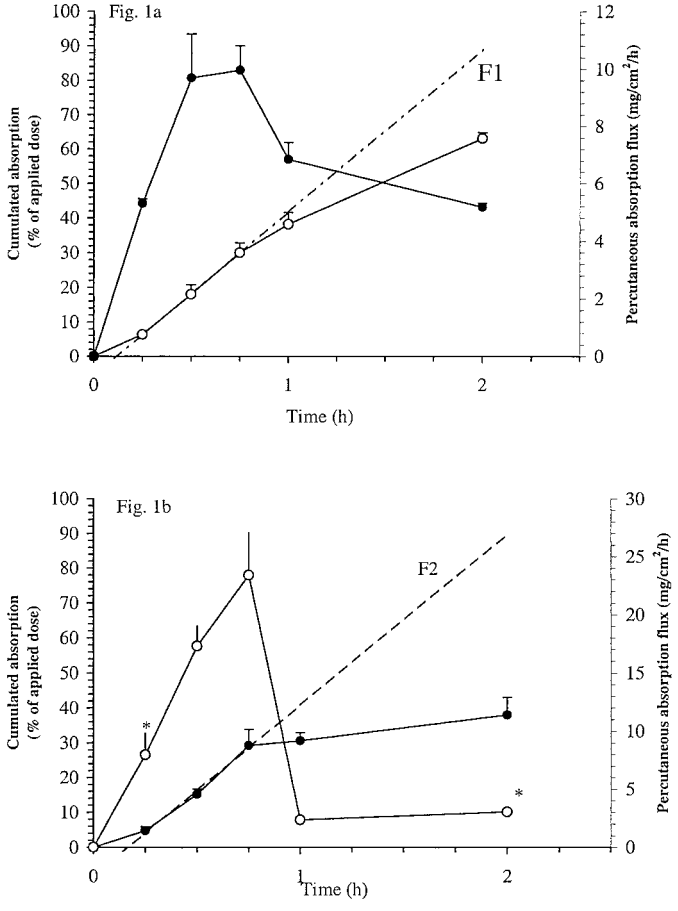

FIG. 1. *In vivo percutaneous absorption after topical application of neat [^{14}C]NMP on male Sprague-Dawley rats.*

Values are expressed as mean ± S.E.M. (n = 4, except for values with an asterisk (*, n = 2). a, cumulated absorption (●) and absorption flux (○) of [^{14}C]NMP (20 μl/cm^2); F1, 9.8 ± 0.1 mg/cm^2/h; T_{lag} = 6.9 ± 0.2 min. b, cumulated absorption (●) and absorption flux (○) of [^{14}C]NMP (40 μl/cm^2); F2, 20.3 ± 1.0 mg/cm^2/h; T_{lag} = 9.8 ± 0.7 min.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

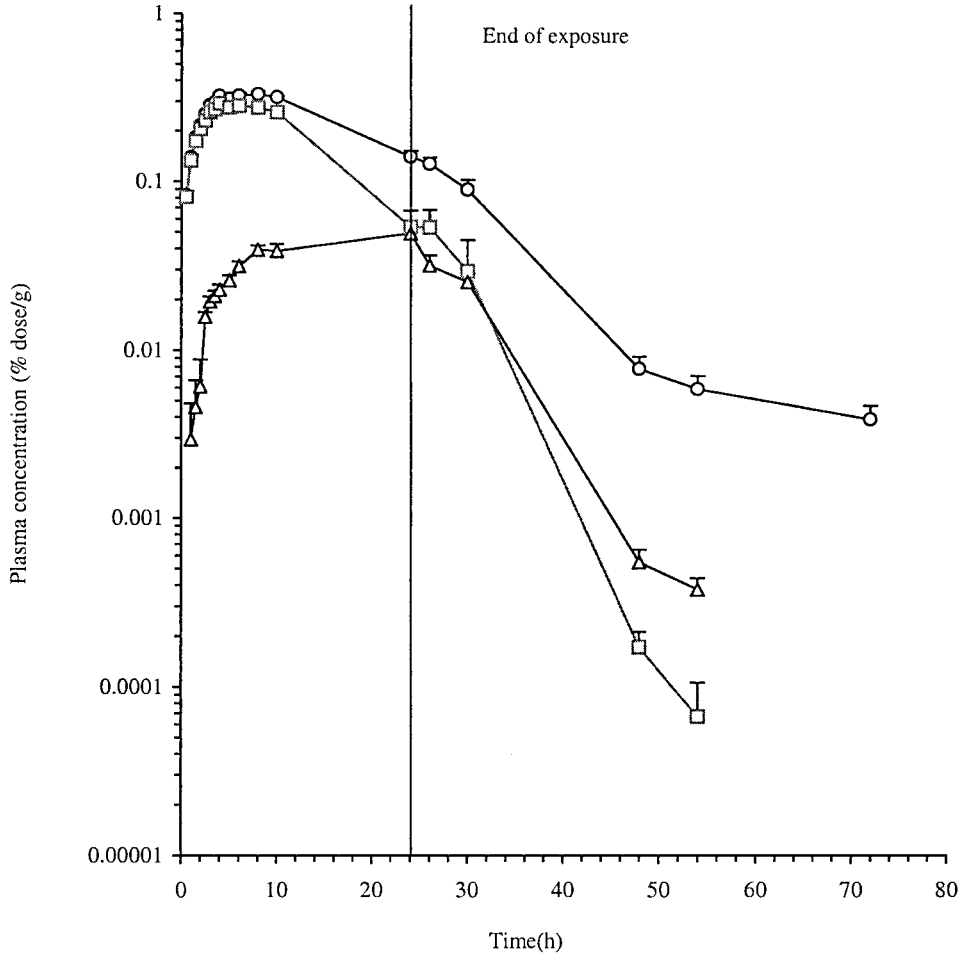

FIG. 2. *Time course of ^{14}C, unchanged NMP, and 5-HNMP after topical application of neat [^{14}C]NMP to male Sprague-Dawley rats.*

Values are expressed as mean ± S.E.M. ($n = 6$). Neat [^{14}C]NMP (20 μl/cm^2, 10 cm^2) was applied to the skin of male rats for 24 h ^{14}C (○), NMP (□), and 5-HNMP (△).

activity in excreta and carcass was 85 ± 4% of the applied dose. An estimation of the absorbed dose, calculated from the ratio of the AUC of NMP and 5-HNMP in plasma after topical application versus an intravenous administration of NMP (500 mg/kg) was overestimated (102%) (Payan et al., 2002). In contrast, the percentage of the absorbed dose calculated from the ratio of urinary excretion of 5-HNMP after topical application versus intravenous administration, was well estimated (87% of the applied dose, result not shown).

In Vitro Skin Metabolism of NMP. After a skin exposure of neat [^{14}C]NMP for 2 h, HPLC profiles of radioactivity of receptor fluids or skin homogenates ($n = 4$) were similar to a standard of [^{14}C]NMP. Radioactivity eluted at a retention time of 5-HNMP standard is barely detectable (result not shown).

Effect of Skin Thickness and Reproducibility on in Vitro Percutaneous Absorption Flux. Skin thickness from shoulder area is significantly higher than that of the skin from haunches area (Table 2). In contrast, the maximal percutaneous absorption fluxes (F_{max}) of neat NMP (25–400 μl/cm^2) are significantly lower in the skin samples from the shoulders than those from the haunches. When the maximal fluxes are normalized for a mean thickness of 1.3 mm (F_{max}Nor), no significant difference can be observed between the fluxes determined with sample skin from the shoulder or the haunches area.

For the same dose of neat NMP (400 μl/cm^2), the coefficients of intra-assay and inter-assay variation for T_{max} and F_{max}Nor are less than 30% (Table 3). No significant differences are observed between the three independent experiments.

Effect of the Topical Dose of Neat NMP on in Vitro Percutaneous Absorption Flux. Figure 4 shows the change in percutaneous absorption fluxes with exposure time after different topical doses of NMP (25–400 μl/cm^2). Whatever the dose of neat NMP, the percutaneous absorption flux increases with time up to a maximum and then decreases gradually following an exponential function. F_{max} Nor and T_{max} increase as the dose of NMP increases. At the highest dose, F_{max} Nor is 7.7 ± 0.2 mg/cm^2/h (Table 4). Variation of F_{max} Nor with the applied dose is well fitted by the classical saturable kinetic model (Fig. 5). The maximal calculated flux for infinite NMP dose was 10.7 ± 0.1 mg/cm^2/h.

Effect of Hydration of the Unabsorbed Dose of NMP on in Vitro Percutaneous Absorption Flux. Neat [^{14}C]NMP with no initial volumic radioactive concentration were applied on skin samples (400 μl/cm^2). Figure 6 represents the change with time of the ratio of volumic radioactivity of non-absorbed NMP versus initial volumic radioactive concentration of NMP and of the absorption flux. The volumic radioactive concentration of unabsorbed NMP decreased with time, which indicated a progressive dilution of the unabsorbed NMP fraction. As previously indicated, the flux of NMP reaches a maximum then gradually declines. In contrast, when the flux is related to the dilution factor of unabsorbed NMP, the flux tends towards a constant value as time goes on.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

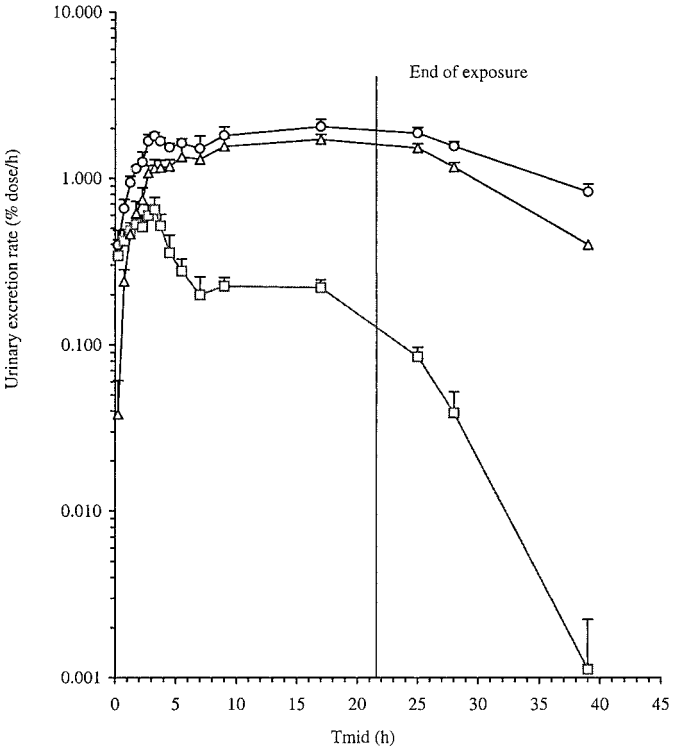

FIG. 3. *Urinary excretion rate of ^{14}C, unchanged NMP, and 5-HNMP after topical application of neat [^{14}C]NMP on male Sprague-Dawley rats.*

Values are expressed as mean ± S.E.M. (n = 6). Neat [^{14}C]NMP (20 μl/cm^2) was applied to the skin (10 cm^2) of male rats for 24 h ^{14}C (○), NMP (□), and 5-HNMP (△). T_{mid}, middle time of the collection period.

Different doses of neat [^{14}C]NMP (100, 200, and 400 μl/cm^2) with the same volumic radioactive concentration where applied on skin. After 4 h of exposure, the volumic radioactive concentrations of unabsorbed NMP and the F_{max} Nor were proportional to the dose (results not shown). In contrast, the F_{max}Nor divided by the dilution factor of the initial concentration of the unabsorbed dose were not significantly different for the three NMP doses tested.

Effect of NMP on the Transfer of ^3H_2O from the Receptor Fluid. Transfer of ^3H_2O from receptor fluid or deposited on skin were similar and accounted 2.5 mg/cm^2 for 4 h of experiment (Table 5). When skin is exposed with neat NMP, the transfer of ^3H_2O from the receptor fluid was more than 10-fold higher (36 mg/cm^2).

Effect of Desquamation and Occlusion on in Vitro Percutaneous Absorption Flux. Occlusion does not affect the percutaneous absorption of neat NMP (Table 6). Desquamation increases F_{max} slightly (+17%) but does not modify T_{max}.

Effect of Aqueous Dilution of NMP on in Vitro Percutaneous Absorption Flux. For different volumes of neat or diluted NMP aqueous solution (1:1 to 1:32, v/v), the relative constant of permeability (F_{max}Nor divided by the NMP concentration) and T_{max} were determined after 24 or 52 h of exposure (Table 7). Except for the lower volume of diluted NMP (25 μl/cm^2), K_{pmax} is independent of the volume of diluted NMP applied on the skin. In contrast, the time to reach maximal percutaneous absorption flux increases as the NMP dilution increases.

Modeling of NMP Transfer. To take into account the transfer of water from the receptor fluid, a simplified mathematical model has been developed (Appendix 1). The "non-dimensional" evolution of the flux (ψ^a) with a non-dimensional time (τ) is defined as a sum of two exponential functions μ and μ_0 are constants without dimension.

$$\psi^a = \frac{\mu_0}{\mu - \mu_0}\left[\exp[-\mu_0(\tau - \tau_0)] - \exp[-\mu(\tau - \tau_0)]\right].$$

The second exponential term corresponds to the decrease of the absorption flux with time after that maximum absorption flux was reached. For a long time, the evolution of the slope of ln $\psi = P(e)$ with the dose of NMP(e) should be able to be expressed by a relationship of the type $P(e) = A/e$; where A is a constant. However, the relation obtained from the experimental data gave a relationship $\ln(P(e) = \ln(0.07) - 0.34 \ln(e)$ which correspond to the approximative relation of $P(e) = P(e) = K/\sqrt[3]{e}$ (Fig. 7).

Discussion

In vivo and in vitro experiments have shown that NMP penetrates the skin of rats rapidly and intensively. Fifteen minutes after topical administration of neat NMP (20 μl/cm^2), the skin contained 2 mg of NMP per square centimeter. This value remained more or less constant for 2 h. The absorbed dose increased linearly with time for 30 min and then declined, although more than 50% of the applied dose was not absorbed. The maximal flux was 10 mg/cm^2/h. A similar decline in flux was also observed 1 h after topical application of 40 μl/cm^2 of neat NMP. The flux calculated was 20 mg/cm^2/h, which is similar to the flux determined in vitro with human skin (Ursin et al., 1995). A very low percutaneous absorption flux (25.3 μg/cm^2/h) was calculated after topical application of a mixture of NMP and 2-pyrrolidone in isopropanol (Midgley et al., 1992). Differences in the dosing conditions could explain the low flux previously reported.

Twenty-four hours after topical application of neat NMP (20 μl/cm^2), 80% of the dose had penetrated into the skin and less than 2% of the applied dose was recovered in the H_2O and CO_2 traps. This result indicates that NMP is intensively absorbed and weakly evaporated. In contrast, 24 h after topical application of neat NMP (10 μl/cm^2) in Sprague-Dawley rats, only 32% of the dose had been

TABLE 2

Effect of the skin thickness of the male rat on the in vitro percutaneous absorption of neat [^{14}C]NMP

Values are given as mean ± S.E.M. (n = 13 rats). The skin of the back of each rat was cut into four parts. Parts 1 and 2 correspond to the skin from the shoulders and parts 3 and 4 correspond to the skin from the haunches. For each animal, parts 1 and 4 or 2 and 3 were tested with the same dose of [^{14}C]NMP (25 to 400 μl/cm^2).

Position Area	Shoulders		Haunches	
	1	2	3	4
Thickness (mm)	1.45 ± 0.04	1.44 ± 0.04	1.24 ± 0.04* ($p < 0.0001$)	1.26 ± 0.04*,** ($p < 0.0001$)
Flux max (mg/cm^2/h)	5.3 ± 0.6	5.6 ± 0.5	6.7 ± 0.7*,** ($p < 0.001$)	6.0 ± 0.7 ($p < 0.0001$)
Flux max Nor (mg/cm^2/h)	5.8 ± 0.6	6.0 ± 0.6	6.3 ± 0.5	5.7 ± 0.7
T_{max} (h)	4.2 ± 0.4	4.2 ± 0.4	3.7 ± 0.4	4.0 ± 0.5

* Values significantly different from area 1.
** Values significantly different from area 2.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

TABLE 3

Intra- and inter-assay reproducibility of the in vitro percutaneous absorption of [^{14}C]NMP in full-thickness rat skin

Values are given as mean ± S.D. (n = 3 rats, two skin samples per rat shoulder and haunch). The skin of the back of each rat was cut into four parts. For each animal neat [^{14}C]NMP (400 μl/cm^2) was deposited on a piece of skin from the shoulder (Sh) and from the haunch (Ha) position.

Weight of Rat (g)	Assay 1 (n = 3)			Assay 2 (n = 3)			Assay 3 (n = 3)			Inter-assay (n = 9)		
	235 ± 7			243 ± 7			226 ± 5			235 ± 7		
	Shoulders	Haunches	Mean (Sh + Ha)	Shoulders	Haunches	Mean (Sh + Ha)	Shoulders	Haunches	Mean (Sh + Ha)	Shoulders	Haunches	Mean (Sh + Ha)
Thickness (mm)	1.34 ± 0.02	1.15 ± 0.08	1.25 ± 0.12 CV = 10%	1.39 ± 0.09	1.28 ± 0.08	1.33 ± 0.1 CV = 8%	1.41 ± 0.12	1.11 ± 0.8*	1.26 ± 0.19 CV = 15%	1.38 ± 0.08	1.18 ± 0.1* ($p < 0.0001$)	1.28 ± 0.3 CV = 20%
F_{max} (mg/cm^2/h)	7.7 ± 0.6	8.1 ± 1.3	7.9 ± 0.9 CV = 11%	6.9 ± 1.1	7.5 ± 0.4	7.2 ± 0.8 CV = 11%	7.3 ± 1.6	8.6 ± 1.3	7.9 ± 1.5 CV = 19%	7.3 ± 1.1	8.1 ± 1.0* ($p < 0.04$)	7.7 ± 1.1 CV = 14%
F_{max} Nor (mg/cm^2/h)	7.2 ± 1.2	8.0 ± 0.7	7.6 ± 1.1 CV = 15%	7.4 ± 0.9	7.4 ± 0.0	7.4 ± 0.6 CV = 8%	7.8 ± 1.4	7.3 ± 0.9	7.6 ± 1.1 CV = 14%	7.7 ± 0.9	7.3 ± 0.8	7.5 ± 1.0 CV = 13%
T_{max} (h)	5.7 ± 0.6	5.7 ± 0.6	5.7 ± 0.5 CV = 9%	6.0 ± 2.0	6.0 ± 2.0	6.0 ± 1.8 CV = 29%	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0 CV < 1%	5.9 ± 1.0	5.9 ± 1.0	5.9 ± 1.2 CV = 20%

* Values significantly different from the shoulder.

absorbed (Bourds et al., 1999). However, in this latter experiment, a large fraction of radioactivity (67%) was found in the charcoal filter covering the skin application site.

The AUC ratio of plasma of total radioactivity, unchanged NMP, or 5-HNMP after topical administration versus an intravenous administration overestimates the absorbed dose. This is probably the result of metabolic saturation of NMP (J.-P. Payan et al., 2002). The maximal plasma concentration of unchanged NMP was higher after topical application than after intravenous administration of the highest NMP dose tested. Similarly, the AUC ratio data of plasma radioactivity after topical administration versus oral dosing an coadministration of NMP and 2-pyrrolidone in rats gave a poor estimation of the absorbed dose (Midgley et al., 1992). In contrast, the total absorbed dose was accurately estimated from the ratio of the urinary excretion of this compound after topical versus intravenous administration.

The decline in flux with time of exposure and the increase of flux with the dose were also observed in vitro. Whatever the dose of neat NMP applied on samples of rat skin, no steady-state percutaneous absorption flux was obtained. In particular, at the highest dose tested (400 μl/cm^2), the percutaneous flux reached a maximum about 6 h after the beginning of exposure and then declined. The decrease in percutaneous flux with exposure time was not the result of a lack of product on the skin. The dose used can be considered as "an infinite dose." At the end of the experiment, 47% of the dose was unabsorbed. It is unlikely the fall in percutaneous was a result of a macroscopic change of the characteristic of the skin as verified by microscopy (results not shown). In contrast, a decrease in the concentration of unabsorbed dose with time correlated well with the decrease in percutaneous absorption flux. The dilution of the unabsorbed NMP resulted from the hygroscopic properties of NMP. The dilution of unabsorbed dose was mainly the consequence of a transfer of the receptor fluid through the skin.

From the evolution of the radioactive concentration of unabsorbed NMP fraction with time of exposure, the transfer rate of water through the skin was calculated to be about 10 mg/cm^2/h for the first 4 h of exposure at the highest dose. A similar value was obtained with the two other doses of neat NMP tested (100 and 200 μl/cm^2). A counter-experiment with ^3H_2O introduced in the receptor fluid confirmed these results. When neat NMP or water was deposited on skin, the transfer rates of ^3H_2O from the receptor fluid were 9 mg/cm^2/h and 0.6 mg/cm^2/h, respectively. This finding indicates that the progressive dilution of the unabsorbed dose of NMP with time was a consequence of the hygroscopic properties of NMP. These results explained the increase in maximal absorption flux and in the time to reach it when the dose increased.

A simplified semiquantitative model of the transfer of NMP through the skin and its dilution over time is proposed. The evolution of the absorption flux with time can be represented by the sum of two exponential functions, which is compatible with the experimental data. However, in this model it is postulated that for a short time of exposure the increase of the absorption flux with time was independent of the doses, which is not fully supported by the experimental data. Moreover, the decline phases of the experimental absorption fluxes were not inversely proportional to the doses as predicted by the mathematical model. This discrepancy can be explained by several reasons that are not taken into account in the mathematical model (e.g., the diffusion coefficient is independent of the skin thickness, no interaction between (water and NMP), and exponential dilution, etc. Also, the transfer of NMP was assumed independent of other substances that migrate. Moreover, it could be possible to make the hypothesis of a value of diffusion coefficient dependent on NMP solvation.

The maximal absorption flux with skin of rats (7.7 mg/cm^2/h) is

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

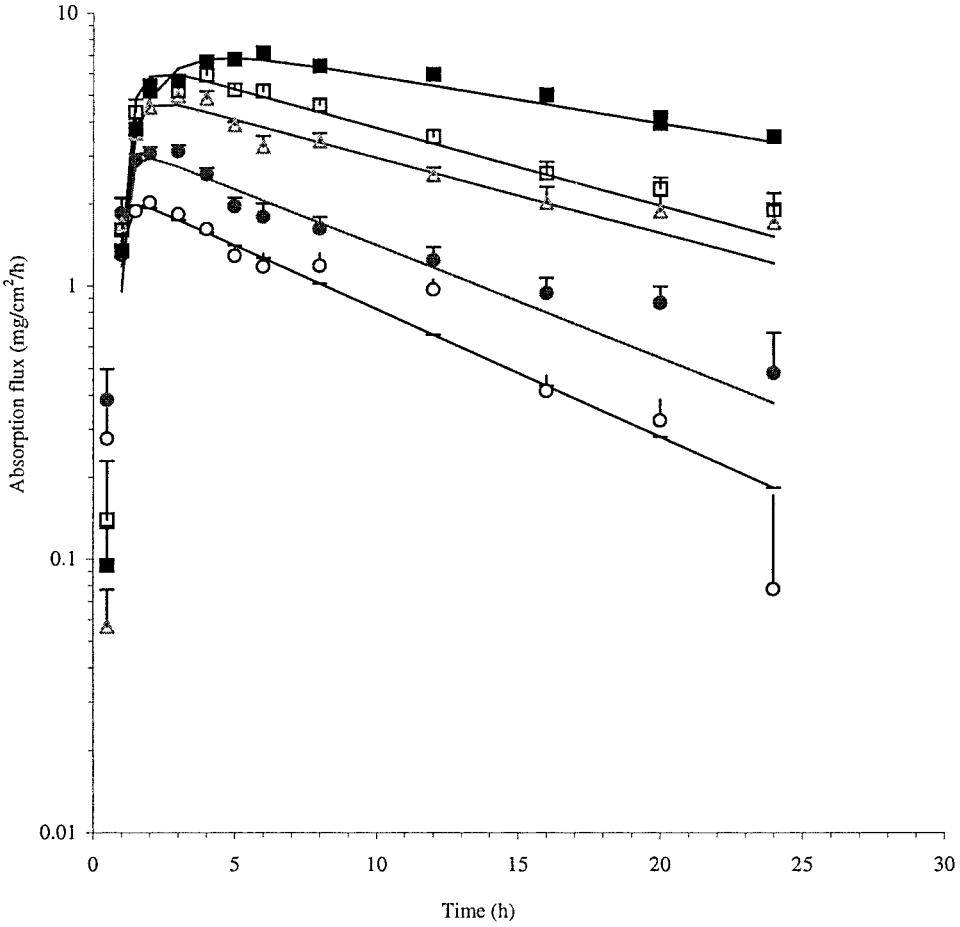

FIG. 4. *Time curve of the in vitro percutaneous absorption flux of neat solutions of NMP.*

Values are expressed as mean ± S.E.M. (n = 4–12). Neat solutions of NMP (25 to 400 μl/cm^2) were applied on full thickness skin of male rats. Percutaneous absorption fluxes are calculated from the radioactivity contained in the receptor fluid after different exposure times. Absorption flux curves are fitted by two exponential functions. The decrease in absorption flux is given by the following equations. 400 μl/cm^2 (■), flux = 8.5 ± 0.3 × exp[(−0.039 ± 0.002 × (t − 0.73 ± 0.08)], n = 12; 200 μl/cm^2 (□), flux = 6.9 ± 0.3 × exp[(−0.065 ± 0.003 × (t − 0.90 ± 0.05)], n = 7; 100 μl/cm^2 (△), flux = 5.3 ± 0.3 × exp[(−0.064 ± 0.007 × (t − 0.92 ± 0.07)], n = 4; 50 μl/cm^2 (●), flux = 3.4 ± 0.2 × exp[(−0.095 ± 0.011 × (t − 0.87 ± 0.09)], n = 6; and 25 μl/cm^2 (○), flux = 2.1 ± 0.0 × exp[(−0.11 ± 0.01 × (t − 0.78 ± 0.01)], n = 6.

TABLE 4

In vitro percutaneous absorption of different doses of neat [^{14}C]NMP into full-thickness skin of male rats

Values are given as mean ± S.E.M. Increasing volumes of neat [^{14}C]NMP were deposited on full thickness skin of male rats. For each rat, a piece of haunch and shoulder was treated with the same volume of NMP.

	25 μl/cm^2 (n = 6)	50 μl/cm^2 (n = 6)	100 μl/cm^2 (n = 4)	200 μl/cm^2 (n = 7)	400 μl/cm^2 (n = 16)
Weight of Rat (g)	240 ± 9	240 ± 9	265 ± 15	247 ± 8	262 ± 11
Thickness (mm)	1.35 ± 0.03	1.23 ± 0.05	1.44 ± 0.09	1.28 ± 0.06	1.29 ± 0.03
Unabsorbed dose (percentage of the dose)	9.5 ± 2.7	10.8 ± 2.3	37.7 ± 2.8[a]	32.5 ± 1.1	47.0 ± 3.4
Skin (mg/cm^2)	4.3 ± 1.1	6.0 ± 0.8		18.7 ± 3.7	28.1 ± 1.8
Recovery (percentage of the dose)	88.1 ± .08	86.6 ± 1.0	88.8 ± 0.8	84.2 ± 4.1	89.5 ± 1.7
F_{max} Nor (mg/cm^2/h)	2.1 ± 0.1	3.4 ± 0.1	5.3 ± 0.3	6.4 ± 0.3	7.5 ± 0.2
T_{max} (h)	1.9 ± 0.2	2.2 ± 0.1	3.4 ± 0.2	3.9 ± 0.3	6.1 ± 0.3

[a] Percentage of the dose in the unabsorbed fraction and in the skin.

lower than the absorption flux determined in vitro in human skin (17 mg/cm^2/h) (Ursin et al., 1997). The difference in flux between the two species may result from skin thickness differences. The thickness of the skin was 0.2 to 0.4 mm and 1.1 to 1.5 mm for the human and rat skin experiments, respectively. It has been shown that, in the rat, the percutaneous absorption flux depends greatly on the thickness of the skin. The absorption flux determined in vitro is lower than that in vivo. The difference in flux between the in vivo and in vitro experiment in rats may be due to the microcirculation, which affects the delivery of drug to the systemic circulation.

From in vitro results, it seems that NMP is not significantly metabolized during its transfer through the skin. Indeed, radioactivity contained in fresh skin or in the receptor fluid after a 2-h exposure of [^{14}C]NMP was predominantly unchanged NMP. Moreover, the absorption flux is dependent on the thickness of the skin and on the concentration of the applied dose of aqueous solution of NMP. Ad-

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

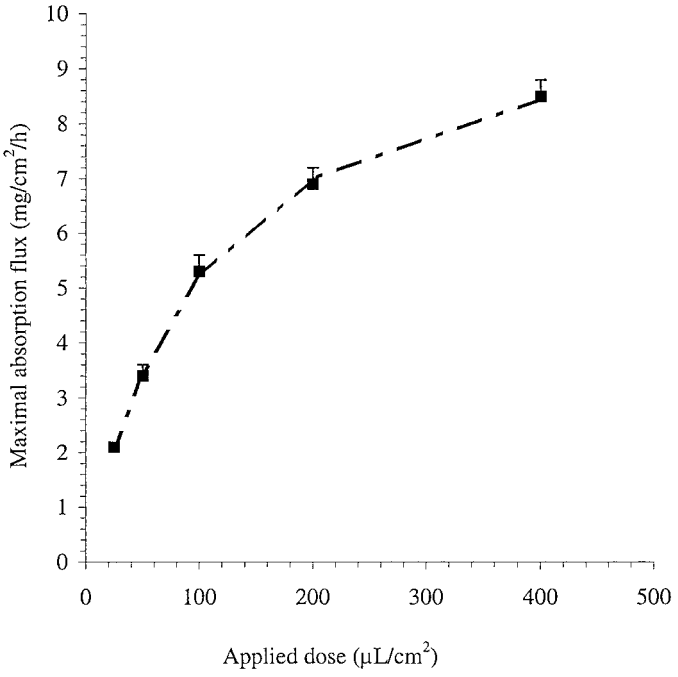

FIG. 5. *Variation of maximal absorption fluxes with the applied doses.*

Values are expressed as mean ± S.E.M. Values were fitted with a non-linear regression model following the classical equation of a saturable kinetic model. The parameters are flux max 10.7 ± 0.1 mg/cm²/h and *K* (NMP dose that leads to have a flux equal to half of flux max = 104 ± 5 µl/cm².

ditionally, skin desquamation only slightly increased the percutaneous absorption flux of NMP. All these findings strongly suggest that the percutaneous absorption of NMP is a passive diffusion process.

In conclusion, NMP is intensively absorbed through the skin in vitro and in vivo in the rat, as indicated in vitro in humans. The hygroscopic properties of this compound affect the process of its absorption drastically.

Appendix 1: Simplified Development Model for the Percutaneous Absorption of NMP Taking into Account the Influence of Water

Model 1. If (*C*) is the concentration of an infinite dose of NMP in contact with the skin and (*L*) the thickness of the latter, the transfer of NMP through the skin in a simple transfer model can be represented as in Fig. 8.

The temporal change (*t*) of *C* can be expressed from the classical following mass transfer equations:

$$C(O) = \text{Co(pure NMP)}$$

$$C(L) = \text{O}$$

$$\frac{\partial c}{\partial t} = D\,\frac{\partial^2 c}{\partial x^2} \qquad \text{(homogeneous Fickian diffusion)}$$

By traditionally expressing

$$\rho = \frac{x}{L} \quad \tau = \frac{Dt}{L^2} \quad \varphi = \frac{C}{\text{Co}}$$

this equation system becomes

$$\varphi(0) = 1$$

$$\varphi(1) = 0$$

$$\frac{\partial \varphi}{\partial \tau} = \frac{\partial^2 \varphi}{\partial \rho^2}$$

and is soluble in the LAPLACE space. By introducing

$$y(s) = \int_O^\infty e^{-s\tau}\varphi(\tau)d\tau$$

this results in

$$\left(\frac{dy}{dp}\right)_1^{\text{model 1}} = \frac{-1}{\sqrt{s}} - \frac{1}{sh(\sqrt{s})}$$

where

$$\left(\frac{dy}{dp}\right)_1^{\text{model 1}}$$

defines the NMP flux for $\rho = 1$ ($x = L$).

$$\left(\frac{dy}{dp}\right)_1^{\text{model 1}}$$

can be reversed to return to real space; the following is obtained

$$\psi_1^o = 1 + 2\sum_{n=1}^{\infty}(-1)^n \exp[-n^2\pi^2\tau]$$

Mathematical development of the homogeneous Fickian diffusion law gives a non-dimensional flux of NMP through the skin, which tends toward an asymptotic unit as time goes on (for review, see Roberts et al., 1999).

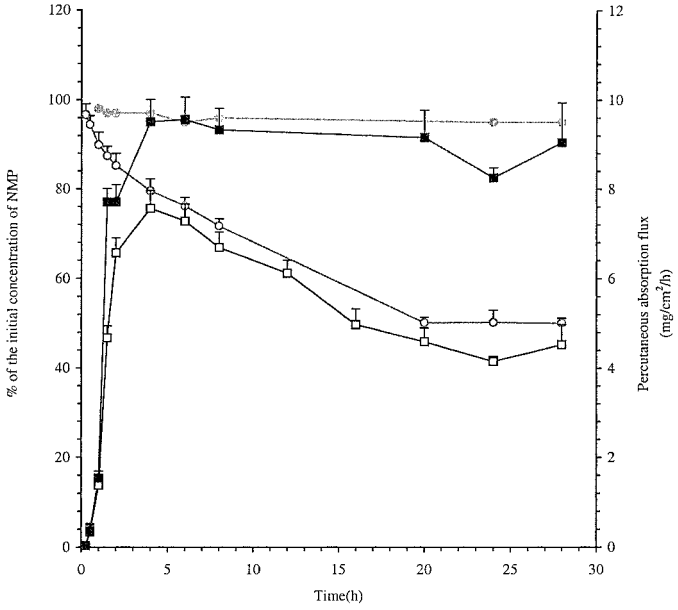

FIG. 6. *Evolution of the concentration of NMP in the donor chamber, percutaneous absorption flux, and corrected flux versus time.*

Values are expressed as mean ± S.E.M. (four rats, two skin samples per rat). Neat NMP (400 µl/cm²) was applied on skin samples (1.76 cm²). An aliquot of receptor fluid (saline solution and RPMI) and unabsorbed NMP fraction (10 µl) as collected at different times. ○, percentage of initial NMP concentration in the unabsorbed fraction deposit on skin; ●, percentage of initial NMP concentration in a separate experiment where neat NMP was simply deposited on watch glass and exposed to the surrounding moistened atmosphere; □, percutaneous absorption flux; ■, percutaneous absorption flux corrected by NMP dilution.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

TABLE 5

Effect of NMP on the transfer of ^3H_2O from the receptor fluid

Values are expressed as mean ± S.E.M. (n = 4). Transfer of ^3H_2O from the receptor fluid was determined from the radioactivity content recovered in the unabsorbed fraction of H_2O or NMP deposit on skin after 4 h of exposure.

Compound deposited on the skin	H_2O	^3H_2O	NMP	[^{14}C]NMP
Receptor fluid	^3H_2O	H_2O	^3H_2O	H_2O
Transfer from receptor fluid (mg/cm^2)	2.5 ± 0.8		36.0 ± 0.4	
Percutaneous absorption of the deposited compound (mg/cm^2)		2.5 ± 0.6		17.2 ± 2.1
Maximal absorption flux (mg/cm^2/h)		1.1 ± 0.2		6.9 ± 0.5

TABLE 6

Occlusion and desquamation effects on in vitro percutaneous absorption of neat NMP into full thickness skin of male rats

Values are expressed as mean ± S.E.M. (n = 3 rat, two skin samples per rat and per modality). Neat NMP was deposited on full thickness skin of male rats for 24 h. F_{max} normalized to a skin thickness of 1.3 mm divided by the concentration of NMP.

		Thickness	Unabsorbed dose	Skin	F_{max} Nor	T_{max}
		mm	*%*	*mg/cm^2*	*mg/cm^2/h*	*h*
Occlusion (hairy rats, n = 3, 400 μl/cm^2)	No	1.3 ± 0.0	46.4 ± 1.4	25.4 ± 2.1	7.4 ± 0.2	5.7 ± 0.8
	Yes	1.2 ± 0.1	43.6 ± 3.1	29.0 ± 3.3	7.2 ± 0.3	5.8 ± 1.0
Desquamation (hairless rats, n = 3, 100 μl/cm^2)	No	1.6 ± 0.0	7.6 ± 1.4	15.7 ± 0.8	4.0 ± 0.3	3.8 ± 0.2
	Yes	1.6 ± 0.0	7.6 ± 1.2	15.1 ± 0.7	4.6 ± 0.1*	3.2 ± 0.2*

* Significantly different from non-desquamated group ($p < 0.05$).

However, for an infinite dose of NMP deposited on skin (400 μl/cm^2), the time curve of the absorption flux reaches a maximum then gradually declines (Fig. 6). In contrast, when the flux is related to the quantity of water (measured by the decline in its initial volumic radioactivity) in the non-absorbed NMP, the flux tends toward a constant value as time goes on. Moreover, the greater the initial quantity of NMP deposited, the higher the maximal absorption flux and the lower the dilution of unabsorbed NMP.

These two results indicated that dilution of the unabsorbed dose from water of the receptor fluid influences the absorption flux of NMP.

Model 2. It was assumed (for reasons of ease of calculation) that the unabsorbed NMP concentration declines as a function of time according an exponential law taking the form:

$$C(o, t) = \mathrm{Co}\exp(-\lambda t)$$

where λ is a constant.

Taking into account the change in the preceding variables, μ is defined by

$$\mu = \frac{\lambda L^2}{D}$$

leading to the following equations.

$$\varphi(0) = \exp(-\mu\tau)$$

$$\varphi(1) = 0$$

TABLE 7

In vitro percutaneous absorption of aqueous dilutions of NMP into full thickness skin of male rats

Values are expressed as mean ± S.E.M. (two skin samples per rat). A volume of 400 μl of neat or aqueous dilution was deposited on full thickness skin of male rats for 24 h or 52 h. K_{pmax} (10^{-3} cm/h) is calculated from F_{max} normalized to a skin thickness of 1.3 mm divided by the concentration of NMP.

Topical Volume	Neat NMP		Dilution 1/2		Dilution 1/8		Dilution 1/16		Dilution 1/32	
	K_{pmax}	T_{max}	K_{pmax}	T_{max}	K_{pmax}	T_{max}	K_{pmax}	T_{max}	K_{pmax}	T_{max}
μl/cm^2										
25	2.1 ± 0.1 (n = 6)	1.9 ± 0.2	2.3 ± 0.2 (n = 3)	5.7 ± 0.3						
			2.3 ± 0.2[a] (n = 3)	8.3 ± 1.9						
50	3.4 ± 0.1 (n = 6)	2.2 ± 0.1								
100	5.3 ± 0.3 (n = 4)	3.4 ± 0.2	5.0 ± 0.1 (n = 4)	17.3 ± 1.3	5.1 ± 1.0 (n = 3)	15.0 ± 3.0				
200	6.4 ± 0.3 (n = 7)	3.9 ± 0.3	5.3 ± 0.1 (n = 3)	22.7 ± 1.3						
			4.3 ± 0.4[a] (n = 4)	25.5 ± 1.5						
400	7.7 ± 0.2 (n = 16)	6.1 ± 0.3	5.1 ± 0.2 (n = 3)	44.7 ± 1.8	7.6 ± 1.2 (n = 3)	35.3 ± 4.1	5.7 ± 1.4 (n = 3)	34.0 ± 8.1	4.7 ± 0.7 (n = 3)	32 ± 2.0
			5.3 ± 0.5[a] (n = 4)	46.5 ± 2.1						
800			4.7 ± 0.5 (n = 4)	49.5 ± 1.0						

[a] Second independent experiment.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

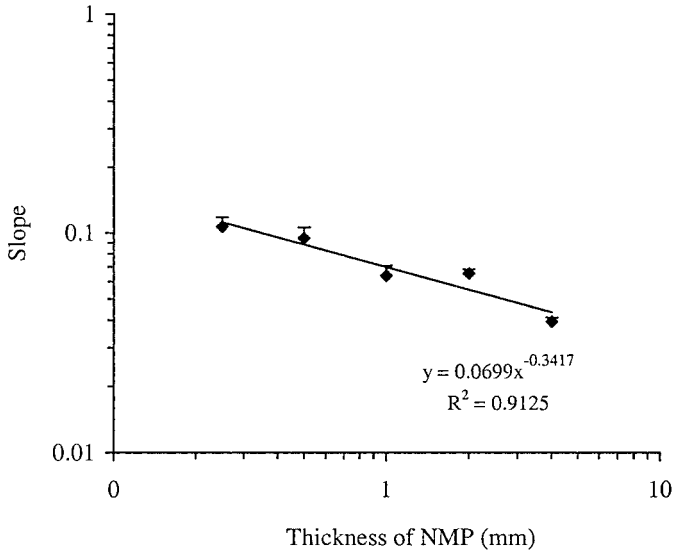

FIG. 7. *Variation in the slope P(e) of the curves of Fig. 4 as a function of the thickness (e) of NMP deposited on the skin.*

The slopes $P(e)$ are determined from the decay phases of the absorption flux evolution curves as a function of time for different thickness of NMP or more exactly for different surface volumes (μl/cm^2) of NMP deposited on the skin samples of constant surface area. $\ln P(e) = -0.34 \ln(e) + \ln(0.07)$; $R = 0.95$; $P(e) \simeq 0.07 \times e^{-1/3}$.

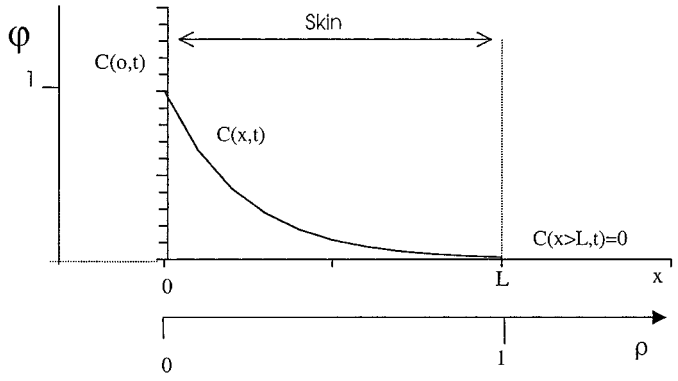

FIG. 8. *Mathematical model transfer of NMP through the skin.*

$$\frac{\partial \varphi}{\partial \tau} = \frac{\partial^2 \varphi}{\partial \rho^2}$$

In these conditions, the expression of the nondimensional LAPLACE flux transform is

$$\left(\left(\frac{\partial \varphi}{\partial \rho} \right)_1 = \psi_1^1 \right), \text{ with } \left(\frac{dy}{d\rho} \right)_1^{\text{model 2}} \text{ defined by:}$$

$$\left(\frac{dy}{d\rho} \right)_1^{\text{model 2}} = -\frac{\sqrt{s}}{sh\sqrt{s}} \cdot \frac{1}{s+\mu}$$

or by

$$\left(\frac{dy}{d\rho} \right)_1^{\text{model 2}} = \left(\frac{dy}{d\rho} \right)_1^{\text{model 1}} \cdot \frac{s}{s+\mu} = \left(\frac{dy}{d\rho} \right)_1^{\text{model 1}} \cdot \left[1 - \frac{\mu}{\mu+s} \right]$$

Bearing in mind that the reverse LAPLACE transform of $\mu/(\mu + s)$ is an exponential [$\mu \exp(-\mu\tau)$], and that the LAPLACE transform of a product is a convolution, it can be considered as a first approximation; with short periods of times:

$$\left(\frac{dy}{d\rho} \right)_1^{\text{model 2}} \cong \left(\frac{dy}{d\rho} \right)_1^{\text{model 1}}$$

Indeed, the dilution of NMP has no time to intervene in the transfer $\psi_1^1 = \psi_1^0$. The curves of Fig. 4 can more or less be superimposed with short periods of time. With long periods of time, it is the influence of dilution that is felt, corresponding to an exponential decline in flux [in $\exp(-\mu\tau)$]. From an experimental point of view, it is indeed what is observed, the flux increasing then declining more or less exponentially. From a mathematical point of view, the nondimensional flux Ψ_1 is defined by the expression:

$$\psi_1^1 = \exp(-\mu\tau)\left[1 + 2\mu \sum_{n=1}^{\infty} \frac{(-1)^n}{\mu - n^2\pi^2} \right]$$

$$- 2 \sum_{n=1}^{\infty} (-1)^n \frac{\mu + n^2\pi^2}{\mu - n^2\pi^2} \exp(-n^2\pi^2\tau)$$

Remark: Simplification of the System. It is possible to represent ψ^0 approximately by:

$$\psi_1^0 = 0 \quad \text{for} \quad \tau \leq \tau_0$$

$$\psi_1^0 = 1 - \exp(-\mu_0(\tau - \tau_0)) \quad \text{for} \quad \tau > \tau_0$$

The parameters τ_0 and μ_0, calculated by a least-square method fitting, respectively, take the values

$$\begin{cases} \tau_0 = 0.065 \\ \mu_0 = 0.40 \end{cases}$$

The LAPLACE transform of this relationship for $\tau > \tau_0$ is

$$z_1^0 = \exp(-s\tau_0)\left[\frac{1}{s} - \frac{1}{s+\mu_0} \right]$$

leading to the transform z_1 of approximated flux ψ^a in the LAPLACE space by

$$z_1 = z_1^0 \cdot \frac{s}{s+\mu}$$

or

$$z = \exp(-s\tau_0) \cdot \frac{\mu_0}{\mu - \mu_0}\left[\frac{1}{s+\mu_0} - \frac{1}{s+\mu} \right]$$

The real image ψ^a is therefore

$$\tau < \tau_0 \qquad \psi^a = 0$$

$$\tau > \tau_0$$

$$\psi^a = \frac{\mu_0}{\mu - \mu_0}[\exp[-\mu_0(\tau - \tau_0)] - \exp[-\mu(\tau - \tau_0)]]$$

corresponding to an approximative biexponential temporal change (which is that observed in Fig. 4). Account taken of the experimental precision, it is this approximated relationship that will be considered in what follows.

With long periods of time, it is the term $\exp[-\mu(\tau - \tau_0)]$ that defines the decline. As Fig. 4 indicates, the relaxation can be considered as more or less exponential, which is in agreement with the experimental data.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018

At the same time, it is possible to determine the slope $P(e)$ corresponding to $\ln\varphi$ of these curves for different values of thickness e of NMP deposited. If the transfer flux of the water is more or less constant, in principle an even more rapid dilution of NMP in the water can be expected as e becomes smaller. In this case $P(e)$ should be able to be expressed by a relationship of the type:

$$P(e)\alpha\frac{A}{e}$$

where A is a constant.

Figure 7 represents the variations in $\ln(P(e))$ versus $\ln(e)$. This highlights a more or less linear experimental relationship taking the form $\ln(P(e)) = \ln K - 0.34 \ln(e)$ where $\ln(K)$ is an arbitrary constant leading to

$$P(e) \cong \frac{K}{\sqrt[3]{e}} \quad \text{with } K = 0.07$$

This experimental result is, however, incompatible with the hypothesis made above.

References

Akesson B and Paulsson K (1997) Experimental exposure of male volunteers to N-methyl-2-pyrrolidone (NMP): acute effects and pharmacokinetics of NMP in plasma and urine. *Occup Environ Med* **54:** 236–240.

Akesson B and Jonsson BA (1997) Major metabolic pathway for N-methyl-2-pyrrolidone in humans. *Drug Metab Dispos* **25:** 267–269.

Barry BW and Bennett L (1987) Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin. *J Pharm Pharmacol* **39:** 535–546.

Bartsch W, Sponer G, Dietmann K, and Fuchs G (1976) Acute toxicity of various solvents in the mouse and rat. LD_{50} of ethanol, diethylacetamide, dimethylformamide, dimethylsulfoxide, glycerine, N-methylpyrrolidone, polyethylene glycol 400, 1,2-propanediol and Tween 20. *Arzneim-Forsch* **26:** 1581.

Bourds SVJ, Griffiths J, McCarthy T, Lorman A, and Parod R (1999) Dermal absorption of N-methylpyrrolidone (NMP) in rat, *38th Annual Meeting of the Society of Toxicology*, New Orleans, LA, 1999, March 14–18; Abstracts, p 72, *Toxicol Sci* 481.

Bower DB (1997) Stillbirth after occupational exposure to N-methyl-2 pyrrolidone. *J Occup Environ Med* **39:** 393–394.

Hass U, Lund SP, and Elsner J (1994) Effects of prenatal exposure to N-methylpyrrolidone on postnatal development and behavior in rats. *Neurotoxicol Teratol* **16:** 241–249.

Midgley I, Hood AJ, Chasseaud LF, Brindley CJ, Baughman S, and Allan G (1992) Percutaneous absorption of co-administered N-methyl-2-[^{14}C]pyrrolidinone and 2-[^{14}C]pyrrolidinone in the rat. *Food Chem Toxicol* **30:** 57–64.

Payan JP, Beydon D, Fabry JP, Boudry I, Cossec B, and Ferrari E (2002) Toxicokinetics and metabolism of N-[^{14}C]methylpyrrolidone in male Sprague-Dawley rats. A saturable NMP elimination process *Drug Metab Disp* **30:** 1418–1424.

Roberts MS, Anissimov YG, and Gonsalvez RA (1999) Mathematical models in percutaneous absorption, in *Percutaneous Absorption Drugs-Cosmetics-Mechanisms-Methodology*, 3rd ed (Bronaugh RL and Maibach HI eds) vol 97, pp 3–55, Drugs and the Pharmaceutical Sciences, Marcel Dekker, Inc., New York.

Solomon HM, Burgess BA, Kennedy GL, and Staples RE (1995) 1-Methyl-2-pyrrolidone (NMP): reproductive and developmental toxicity study by inhalation in the rat. *Drug Chem Toxicol* **18:** 271–293.

Sugibayashi K, Sakanoue C, and Morimoto, Y (1989) Utility of topical formulation of morphine hydrochloride containing azone and N-methylpyrrolidone. *Sel Cancer Ther* **5:** 119–128.

Ursin C, Hansen CM, Van Dyk JW, Jensen PO, Christensen IJ, and Ebbehoej J (1995) Permeability of commercial solvents through living human skin. *Am Ind Hyg Assoc J* **56:** 651–660.

Wells DA, Hawi AA, and Digenis GA (1992) Isolation and identification of the major urinary metabolite of N-methylpyrrolidone in the rat. *Drug Metab Dispos* **20:** 124–126.

Downloaded from dmd.aspetjournals.org at ASPET Journals on March 21, 2018