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TISSUE DISTRIBUTION AND EXCRETION OF N-METHYL-2-PYRROLIDONE IN MALE AND FEMALE RATS

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Abstract
Objectives: N-methyl-2-pyrrolidone (NMP) belongs to solvents widely used in the petrochemical industry a well as in the production of pesticides, veterinary drugs and paint removers. NMP is easily absorbed from the respiratory tract, digestive system and through the skin. It is a compound of slight acute toxicity that also displays moderate irritating activity. The aim of this study was to assess tissue distribution and excretion following a single intraperitoneal NMP administration. **Materials and Methods:** Tissue distribution and excretion of NMP following administration of a single dose of 250 mg/kg body weight (350 kBq/rat) was investigated using ^{14}C. Blood plasma (6 rats per time point) were sampled up to 72 h after administration and determination of radioactivity. Male and female rats (4 animals per time point) were decapitated at appropriate time intervals and examined tissues were removed for determination of radioactivity. Excretion of ^{14}C in urine and feces were also measured. All radioactivity measurements were carried out using a Rackbetta 1209 (LKB, Sweden) liquid scintillation counter. **Results:** The highest ^{14}C activity in tissues and internal organs of female and male rats was observed 4 h after administration of the compound. The highest accumulation was detected in the muscles and fat tissue as well as in the liver and testicles. During 72 h following administration, approximately 80% of the dose was excreted in urine. Elimination of the compound in feces was far less significant: only about 5% of the dose was excreted at once. **Conclusions:** The results of the study indicate that there are no significant differences in ^{14}C-NMP tissue distribution between male and female rats; NMP absorption from the peritoneal cavity to blood is rapid, disappearance from plasma is monophase and kidneys are the main route of excretion of NMP and/or its metabolites from the rat body after administration of a dose equal to 10% of LD_{50}. The ability to accumulate NMP and/or its metabolites in testes and seminal vesicles may be the reason for fertility impairment in male rats observed after repeated exposure to this compound.

Key words:
N-methyl-2-pyrrolidone, Tissue distribution, Excretion, Rats

INTRODUCTION

N-methyl-2-pyrrolidone (NMP) is a solvent widely used in catalysis of polymerization processes in the petrochemical industry as an extraction agent and in the production of herbicides, insecticides, pigments, cosmetics, and drugs. NMP shows low toxicity in rats (oral LD_{50} is about 4.0 g/kg of body weight (b.w.)) [1,2,3] and it is readily absorbed through the skin, respiratory and gastrointestinal tracts [4,5,6].

N-methyl-2-pyrrolidone is eliminated from the body mainly by biotransformation to polar compounds, which are excreted in urine [7,8]. The results of experimental studies indicate that NMP induces some developmental toxic ef-

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fects in prenatally exposed offspring [2,9,10]. It is neither genotoxic nor mutagenic [11,12,13].

The aim of the present study was to assess the kinetics of body distribution and excretion of NMP in male and female rats following its single intraperitoneal (i.p.) administration.

The analysis of data concerning NMP tissue distribution, particularly its possible deposits in the animal reproductive system, may help explain the toxic effect of this compound on fertility.

MATERIALS AND METHODS

Chemicals

Radiolabeled N-[^{14}C]methyl-2-pyrrolidone ([^{14}C]-NMP) with specific activity of about 3.2 mCi/g was purchased from the Department of Radiochemistry (Institute of Radiation Technique, Technical University of Łódź, Poland). Purity was determined by HPLC, and made up 98.6% of total chromatogram radioactivity. N-methyl-2-pyrrolidone (CAS 872-50-4) of analytical grade (Sigma, Aldrich, USA) was used for dilution of radioactive samples. The liquid scintillator Ekoluma (Baker, Deventer, The Netherdands), perchloric acid and perhydrol were purchased from Merck, Germany. All other chemicals were of analytical grade.

Animals and administration

After 7 days of adaptation, 26 male and 20 female rats approximately 14 weeks of age and 325–350 g (males) and 210–220 g (females) of b.w. obtained from our own breeding colony Imp: WIST were used in the study. The animals were housed in cages with controlled temperature of 22 ± 1°C, a light/dark cycle of 12/12 h (light on at 6:00), and relative humidity of 55–60%. They were maintained on commercial pelleted chow (Fodder Factory, Motycz, Poland) and had free access to tap water.

The rats were put individually in the glass metabolism cages (Simax, the former Czechoslovakia), acclimatized for 48 h, and subsequently administered i.p. ^{14}C-NMP. Male and female rats were given i.p. a single dose of ^{14}C-N-methyl-2-pyrrolidone dissolved in 0.9% NaCl (250 mg/kg b.w., 350 kBq/rat). This dose is equal to 10% of LD_{50} i.p. dose for rats. Each animal received 0.5 ml of the solution. Immediately after administration, the rats were placed in metabolism cages, which allowed to collect samples of urine and feces.

In all the experiments, the Polish law on the protection of animals was followed [14]. The study design was approved by the Local Ethic Committee in Łódź (No. Ł/BD/140, 29.07.2002)

Sampling of biological material and measurements of ^{14}C-radioactivity

Blood samples (0.03 ml) were collected from the tail veins of six rats using calibrated, heparinized capillaries (0–72 h) following a single i.p. administration of ^{14}C-NMP.

The animals were decapitated under light ether narcosis and tissues were collected for radioactivity determination. The liver, kidneys, lungs, brain, spleen, testicles, epididymis, and seminal vesicles were homogenized. The sciatic nerve, adrenals, ovaries, uterus, a piece of abdominal fat, and a piece of muscular tissue from muscle quadriceps-femoral were digested directly.

All tissue homogenates (25% in water), and feces (10% water homogenates) and erythrocytes were digested according to Mahin and Lofberg [15] before radioactivity measurement. Each time, two parallel samples were collected for determination. The remaining tissue samples, plasma and urine (diluted with water to the volume of 50 ml), were directly measured. The radioactivity of samples, placed in glass scintillation vials with 15 ml of the scintillator, was measured with a Rackbetta 1209 (LKB Sweden) liquid scintillation counter. Counting correction was archived using the external standard method.

The results were analyzed with Excel 7.0 (Microsoft Corporation) computer program. The kinetics of ^{14}C activity in plasma were carried out using SIGMA PLOT 3.0 (Jandel Corporation) for WINDOWS.

RESULTS

The kinetics of increase and decrease in ^{14}C radioactivity in the serum of male rats between 15 min and 72 h following ^{14}C-NMP administration is presented in Fig. 1. Maximum

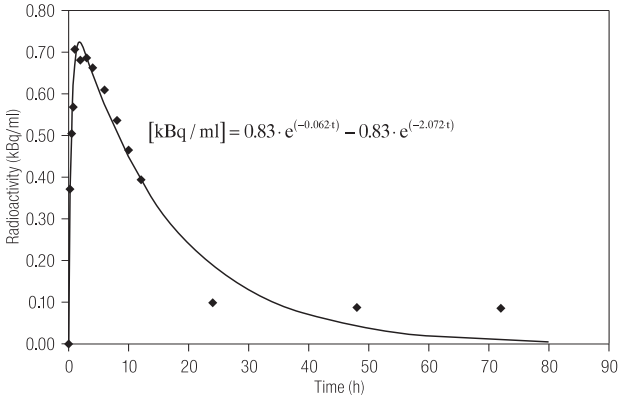

Fig 1. Kinetics of ^{14}C in blood plasma after single i.p. administration of ^{14}C-N-methyl-2-pyrrolidone at a dose of 250 mg/kg b.w. (350 kBq/animal) in male rats. Results are means from 6 male rats ± SD.

radioactivity of ^{14}C in serum was observed between 45 min and 4 h after the exposure. A decrease in radioactivity was detected after 6 h, and 24 h after administration it was very low. The kinetics of absorption and decline of ^{14}C in serum was described in the following equation:

$$[A] = 0.83 \cdot e^{(-0.062 \cdot t)} - 0.83 \cdot e^{(-2.072 \cdot t)}$$

The accretion of ^{14}C proceeded with kinetic constant = 2.072 h. The decline in serum ^{14}C was monophase with kinetic constant = 0.062, while calculated half time of ^{14}C decline was approximately 11 h. The dynamics of ^{14}C-NMP distribution in selected organs and tissues of the rats administered i.p. the compound at a dose of 250 mg/kg body weight (350 kBq/rat) is displayed in Tables 1 (males) and 2 (females). The highest level of ^{14}C activity calculated for 1g of tissue in male rats was detected 4 h after administration in adrenals, kidneys, seminal vesicles, testes, muscles, liver, brain, and lungs.

In the female rats, however, the highest activity was detected in muscles, kidneys, lungs, ovaries, brain, sciatic nerve, adrenals and liver also 4 h following the administration.

The activity of ^{14}C decreased over time in all tissues and organs of both male and female rats. It is worth pointing out that ^{14}C activity in female fat tissue was higher 8 h after administration than 4 h after exposure.

Total amount of ^{14}C retained in tissues during 72 h after ^{14}C-NMP administration expressed as the percentage of the dose is presented in Tables 3 and 4. In all examined tissues, a decrease in ^{14}C activity was detected in subsequent time points. The highest radioactivity was observed in muscles after 72 h.

Excretion and total amount of ^{14}C retained in the organism of male and female rats after a single i.p. dose of ^{14}C-NMP are illustrated in Tables 5 and 6. The main route of ^{14}C-NMP excretion was the urinary tract. As depicted in the tables, more than 60% of the administered dose was

Table 1. Radioactivity concentration in different male rat tissues after administration of ^{14}C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/male)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.11 ± 0.11[a]	0.89 ± 0.01	0.05 ± 0.01	0.04 ± 0.001	0.04 ± 0.001
Kidneys	1.40 ± 0.15	1.08 ± 0.02	0.11 ± 0.02	0.04 ± 0.001	0.04 ± 0.001
Spleen	0.69 ± 0.10	0.67 ± 0.10	0.05 ± 0.01	0.03 ± 0.002	0.03 ± 0.0007
Lungs	1.04 ± 0.13	0.86 ± 0.14	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.002
Brain	1.05 ± 0.11	0.91 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.0007
Adrenals	1.42 ± 0.10	1.03 ± 0.10	0.07 ± 0.01	0.06 ± 0.001	0.05 ± 0.0007
Sciatic nerve	0.96 ± 0.04	1.04 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.007
Muscles	1.32 ± 0.19	1.44 ± 0.01	0.03 ± 0.001	0.07 ± 0.004	0.02 ± 0.002
Fat	0.83 ± 0.30	0.67 ± 0.10	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.001
Epididymis	0.89 ± 0.08	0.82 ± 0.02	0.05 ± 0.02	0.04 ± 0.005	0.03 ± 0.003
Testicles	1.28 ± 0.14	1.08 ± 0.009	0.05 ± 0.02	0.03 ± 0.005	0.03 ± 0.001
Seminal vesicles	1.33 ± 0.14	0.99 ± 0.08	0.09 ± 0.07	0.04 ± 0.003	0.02 ± 0.002
Whole blood	0.87 ± 0.04	0.70 ± 0.05	0.18 ± 0.003	0.17 ± 0.01	0.18 ± 0.01

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 male rats at each time.
To assess ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12%, and muscles as 40% of whole b.w. [17].

Table 2. Concentration of radioactivity in different female rat tissues after administration of ^{14}C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/female)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.45 ± 0.16[a]	0.88 ± 0.03	0.06 ± 0.01	0.04 ± 0.005	0.04 ± 0.001
Kidneys	1.70 ± 0.14	1.16 ± 0.22	0.09 ± 0.01	0.05 ± 0.002	0.06 ± 0.007
Spleen	0.87 ± 0.10	0.47 ± 0.05	0.05 ± 0.009	0.04 ± 0.002	0.03 ± 0.004
Lungs	1.70 ± 0.16	1.23 ± 0.06	0.06 ± 0.01	0.05 ± 0.001	0.05 ± 0.002
Brain	1.59 ± 0.19	1.14 ± 0.19	0.02 ± 0.002	0.02 ± 0.002	0.03 ± 0.0007
Adrenals	1.48 ± 0.18	0.98 ± 0.10	0.06 ± 0.005	0.06 ± 0.008	0.05 ± 0.003
Sciatic nerve	1.57 ± 0.16	1.52 ± 0.27	0.05 ± 0.007	0.05 ± 0.003	0.05 ± 0.0007
Muscles	2.02 ± 0.17	1.48 ± 0.12	0.04 ± 0.001	0.07 ± 0.004	0.04 ± 0.007
Fat	0.43 ± 0.08	0.82 ± 0.26	0.06 ± 0.006	0.06 ± 0.004	0.03 ± 0.006
Ovaries	1.66 ± 0.20	0.93 ± 0.16	0.05 ± 0.01	0.05 ± 0.008	0.04 ± 0.005
Uterus	1.19 ± 0.09	0.94 ± 0.03	0.04 ± 0.01	0.04 ± 0.005	0.04 ± 0.007

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 female rats at each time.
To assess ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of whole b. w. [17].

Table 3. Total balance sheet of ^{14}C radioactivity in male rats after single i.p. administration of ^{14}C-N-methyl-2-pyrrolidone at a dose of 250 mg/kg b.w. (350 kBq/male)

Tissue	0–4 h	0–8 h	0–24 h	0–48 h	0–72 h
	Percent of dose administration				
Liver	4.13 ± 0.72[a]	3.14 ± 0.23	0.20 ± 0.05	0.18 ± 0.03	0.16 ± 0.01
Kidneys	0.90 ± 0.11	0.66 ± 0.03	0.07 ± 0.03	0.03 ± 0.005	0.03 ± 0.005
Spleen	0.13 ± 0.0009	0.13 ± 0.04	0.08 ± 0.002	0.008 ± 0.001	0.006 ± 0.001
Lungs	0.53 ± 0.03	0.47 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.002
Brain	0.59 ± 0.03	0.54 ± 0.0005	0.02 ± 0.005	0.015 ± 0.002	0.01 ± 0.001
Adrenals	0.008 ± 0.001	0.03 ± 0.02	0.001 ± 0.0001	0.0009 ± 0.0001	0.0006 ± 0.0001
Sciatic nerve	0.01 ± 0.002	0.01 ± 0.002	0.001 ± 0.0002	0.0006 ± 0	0.0006 ± 0
Muscles	59.98 ± 8.72	63.49 ± 0.68	1.05 ± 0.53	3.53 ± 0.58	1.19 ± 0.14
Fat	11.15 ± 4.58	8.89 ± 1.34	0.54 ± 0.15	0.86 ± 0.29	0.32 ± 0.02
Epididymis	0.23 ± 0.02	0.18 ± 0.01	0.01 ± 0.002	0.01 ± 0.002	0.008 ± 0.0006
Testicles	1.36 ± 0.005	1.06 ± 0.08	0.05 ± 0.01	0.04 ± 0.003	0.03 ± 0.003
Seminal vesicles	0.69 ± 0.08	0.55 ± 0.06	0.05 ± 0.03	0.02 ± 0.002	0.02 ± 0.001
Whole blood	6.39 ± 0.70	5.26 ± 0.48	1.37 ± 0.18	1.28 ± 0.09	1.31 ± 0.08
Total	86.13	84.37	3.41	6.00	3.10

i.p. – intraperitoneally; b.w. – body weight; a – the value presented as the percent of ^{14}C-N-methyl-2-pyrrolidone dose from 4 male rats at each time.
To asses ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12%, and muscles as 40% of the whole b.w. [17]

eliminated in urine, and only about 2–2.5% in feces during the first 24 h. (Tables 5 and 6). About 83% of the dose was detected in male and female excrements 72 h after i.p. administration, while the total amount of ^{14}C retained in tissues and excreted from the organism was 85–86%. Muscles played an important role in retaining ^{14}C, which results from their biggest mass.

DISCUSSION

The results of the presented experiment suggest that ^{14}C-NMP administered intraperitoneally in a single dose to female and male rats is rapidly absorbed into blood, but also quickly eliminated from the body. A similar profile of the NMP concentration increase and decrease in the blood serum was observed in rats after its intragastrical

Table 4. Total balance sheet of ^{14}C radioactivity in female rats after single i.p. administration of ^{14}C-N-methyl-2-pyrrolidone at a dose of 250 mg/kg b.w. (350 kBq/female)

Tissue	0–4 h	0–8 h	0–24 h	0–48 h	0–72 h
	Percent of dose administered				
Liver	3.75 ± 0.23[a]	2.21 ± 0.34	0.15 ± 0.02	0.14 ± 0.01	0.12 ± 0.01
Kidneys	0.72 ± 0.07	0.49 ± 0.08	0.04 ± 0.003	0.02 ± 0.002	0.03 ± 0.003
Spleen	0.16 ± 0.01	0.08 ± 0.001	0.01 ± 0.001	0.006 ± 0.0001	0.006 ± 0.0001
Lungs	0.69 ± 0.10	0.55 ± 0.04	0.03 ± 0.002	0.02 ± 0.001	0.02 ± 0.001
Brain	0.84 ± 0.05	0.58 ± 0.13	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
Adrenals	0.02 ± 0.004	0.02 ± 0.001	0.001 ± 0	0.001 ± 0	0.001 ± 0
Sciatic nerve	0.01 ± 0.005	0.01 ± 0.005	0.001 ± 0	0.0006 ± 0	0.0006 ± 0
Muscles	57.71 ± 5.11	54.56 ± 3.91	1.30 ± 0.41	2.19 ± 0.51	1.27 ± 0.23
Fat	3.70 ± 0.41	7.28 ± 0.64	0.52 ± 0.06	0.62 ± 0.05	0.31 ± 0.05
Ovaries	0.04 ± 0.001	0.03 ± 0.0009	0.002 ± 0	0.002 ± 0	0.002 ± 0
Uterus	0.13 ± 0.006	0.12 ± 0.001	0.006 ± 0	0.006 ± 0	0.003 ± 0
Total	67.83	65.95	2.07	3.03	1.77

i.p. – intraperitoneally; b.w. – body weight; a – the value presented as the percent of ^{14}C-N-methyl-2-pyrrolidone dose from 4 female rats at each time.
To asses ^{14}C distribution, fat tissue was accepted as 12% and muscles as 40% of the whole b.w. [17].

Table 5. Total balance of ^{14}C in male rats following single i.p. administration of ^{14}C-N-methyl-2-pyrrolidone at a dose of 250 mg/kg b.w. (350 kBq/male)

Medium	Percent of administered dose		
	0–24 h	0–48 h	0–72 h
Urine	65.26 ± 7.15[a]	77.13 ± 4.18	78.45 ± 5.67
Feces	2.00 ± 1.34	3.63 ± 0.96	4.45 ± 2.08
Whole blood	1.37 ± 0.03	1.28 ± 0.01	1.31 ± 0.01
Fat	0.54 ± 0.15	0.86 ± 0.29	0.32 ± 0.02
Muscles	1.05 ± 0.53	3.53 ± 0.58	1.19 ± 0.14
Liver	0.20 ± 0.05	0.18 ± 0.03	0.16 ± 0.01
Remaining tissues	0.25	0.14	0.11
Total	71.41	86.74	86.01

i.p. – intraperitoneally; b.w. – body weight;
a – the value presented as the percent of ^{14}C-N-methyl-2-pyrrolidone dose from 4 male rats at each time.
To asses ^{14}C distribution blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of the whole b.w. [17].

Table 6. Total balance of ^{14}C in female rats following single i.p. administration of ^{14}C-N-methyl-2-pyrrolidone at a dose of 250 mg/kg b.w. (350 kBq/female)

Medium	Percent of administered dose		
	0–24 h	0–48 h	0–72 h
Urine	63.61 ± 8.98[a]	74.81 ± 5.04	78.00 ± 7.45
Feces	2.51 ± 0.67	3.48 ± 0.64	5.43 ± 1.39
Fat	0.52 ± 0.06	0.62 ± 0.05	0.31 ± 0.05
Muscles	1.30 ± 0.41	2.19 ± 0.51	1.27 ± 0.23
Liver	0.15 ± 0.02	0.14 ± 0.01	0.12 ± 0.01
Remaining tissues	0.10	0.066	0.072
Total	68.19	81.31	85.19

i.p. – intraperitoneally; b.w. – body weight;
a – the value presented as the percent of ^{14}C-N-methyl-2-pyrrolidone dose from 4 female rats at each time.
To asses ^{14}C distribution blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of the whole b.w. [17].

administration. The highest serum NMP concentration was detected 2 h after its administration to the animals [18]. As a result of a 6 h/day inhalation exposure of rats to NMP at a concentration of 618 mg/m^3, the increase in this compound was detected in the blood of animals 4 h after the exposure cessation [6].

However, after intravenous administration of NMP, its rapid increase in tissues was observed. The highest ^{14}C-NMP radioactivity was measured in the liver, small and large intestines, testes, stomach and kidneys 6 h following the injection, but 24 h after injection, it was detected only in the liver and intestines [19]. The maximum NMP concentration in the blood of human volunteers was observed 3 h after applying the compound on the skin at a dose of 300 mg, whereas over 20% of the dose applied was detected in their urine as unchanged or metabolized substance [20].

The analyzed blood concentration of NMP metabolites in rats administered ^{14}C intravenously suggests that the main NMP metabolite, 5-hydroxy-N-methylpyrrolidone (5-HNMP), reaches maximum concentration also between 4 and 6 h following the injection of NMP at doses ranging from 0.1 to 10 mg/kg. But after intravenous administration of higher doses (100 and 500 mg/kg) a similar effect can be observed much later, i.e. between 8 and 24 h [21].

The assessment of NMP excretion performed in this study suggests that urinary tract is the main route of elimination of the compound itself and its metabolites from the body of female and male rats following a single i.p. administration of ^{14}C-NMP at a dose of 250 mg/kg b.w. During the first 24 h after administration, approximately 65% of the substance was excreted via this route, while during 72 h of the experiment about 80% of the dose was eliminated in urine. During the same time (72 h) only about 5% of NMP dose was excreted in feces. Similar dynamics and routes of NMP excretion were observed by other authors in male volunteers who were administered 100 mg of NMP orally [7] as well as in animals administered this compound intravenously or intragastrically [19,21,22].

Relatively high concentration of ^{14}C in testes, seminal vesicles as well as in ovaries and uterus observed in our study seems to be worth considering. Although NMP and its metabolites are quickly eliminated from the body, it can be assumed that repeated exposure to this compound may have caused disturbances in spermatogenesis and impairment of fertility in male rats as well as disturbances in estrus cycle in female rats observed in fertility and gonadotoxicity tests in rats administered NMP *per os* for 11 weeks [23]. Testicular degeneration and atrophy were also observed in male rats given NMP in the diet for 28 days [24].

In summary, the presented study indicates that there are no significant differences in tissue distribution of ^{14}C-NMP between male and female rats. N-methyl-2-pyrrolidone is readily absorbed and rapidly excreted from their bodies following i.p. injection at a dose of 250 mg/kg, but its relatively high concentration in sexual organs may induce fertility disturbances in rats after repeated exposure.

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