

Original Article

Evaluation of Reproductive Disorders in Female Rats Exposed to *N*-Methyl-2-Pyrrolidone

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BACKGROUND: *N*-methyl-2-pyrrolidone (NMP) is a solvent used in the petrochemical, and electronic industries, in pesticides production, veterinary drugs, and paint removers. The aim of study was to evaluate the relationship between the dose of NMP given orally and its effect on fertility in female rats and early development of their progeny. **METHODS:** Females were exposed by gavage 5 days/week to NMP at 150, 450, or 1000 mg/kg/day 2 weeks before mating, during mating, gestation, and lactation. On the first postnatal day (PND 1), the live and dead pups were counted, weighed, and gender was determined. On PND 4, the litters were culled to eight animals each and balanced for gender. Young animals were observed during 3 weeks after birth. **RESULTS AND CONCLUSION:** Fertility index did not significantly differ in the control and the group exposed at 150 mg/kg/day but it was significantly lower in the groups exposed at 450 or 1000 mg/kg/day. The number of live pups in the group exposed to the highest dose was significantly lower and the number of stillbirths in litters was significantly greater. Survival of the pups from all exposed groups during the 3 weeks after birth was significantly lower than the control animals. The results of our study indicate that intragastric exposure of female rats to NMP before pregnancy during gestation causes significant impairment in female fertility and intrauterine mortality rates. At lower doses, toxic or slightly toxic to the mothers, this substance causes decrease in viability and physical development of progeny. *Birth Defects Res (Part B)* 95:195–201, 2012. © 2012 Wiley Periodicals, Inc.

Key words: *N*-methyl-2-pyrrolidone; NMP; CAS 872-50-4; female rats; fertility

INTRODUCTION

N-methyl-2-pyrrolidone (NMP) is a solvent easily miscible with water and used in the petrochemical and chemical industries for the production of veterinary drugs, pigments, herbicides, and cosmetics. NMP also has numerous applications in the electric and electronic industries. Considering good solubility both in polar and nonpolar solvents, NMP may be expected to easily permeate the biological membranes (Becci et al., 1982; Ursin et al., 1995; Payan et al., 2003; Bader et al., 2005; Keener et al., 2007). Values of median lethal doses LD₅₀ following oral administration to rats, mice, guinea pigs, and rabbits range from 3900 to 7700 mg/kg (Ansell and Fowler, 1988), while median lethal doses after its application to the skin of rats or rabbits range from 4000 to 10,000 mg/kg/day (Bartsch et al., 1976). The results of experimental studies indicate that NMP induces some developmental toxic effects in prenatally exposed offspring (Becci et al., 1982; Hass et al., 1994; Solomon et al., 1995). Increased preimplantation losses, delayed ossification, and delay of postnatal development were observed in the offspring of female rats exposed to NMP during gestation (Hass et al., 1995, 1994).

NMP induced skeletal and visceral malformations in the fetuses prenatally exposed orally at doses toxic to

the mothers; but at lower doses, nontoxic to pregnant females, NMP was fetotoxic (Saillenfait et al., 2002). In two-generation study on rats exposed to NMP by inhalation, the parameters of reproductive performance for exposed and control rats were similar (Solomon et al., 1995). Observations of occupational exposure to NMP and other chemicals (methanol, acetone) in 23-year-old laboratory technicians who were exposed by inhalation and dermal route during first 20 weeks of pregnancy are reported in a study by Solomon et al. (1996). In the medical examination performed in women at 25 weeks of gestation, there were signs of delayed fetal development, and at 31 weeks a stillborn fetus was delivered. Because the concentration of NMP in the air was unknown, it is difficult to conclude that exposure to this substance was the cause of stillbirths (Bower, 1997; Solomon et al., 1997).

Subchronic oral exposure of male rats to this chemical produces gonadotoxic effect and brings about infertility (Sitarek and Stetkiewicz, 2008).

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NMP is eliminated from the body mainly by biotransformation to polar compounds. The main route of NMP and its metabolite excretion is the urinary tract, more than 60% of dose of rats was eliminated in urine and approximately 2% in feces during the first 24 hr after i.p. administration. However, 6 hr after intravenous injection of NMP, the highest ^{14}C -NMP radioactivity was detected in the liver, intestine, testes, stomach, and kidney, but 24 hr after injection it was measured only in liver and intestines (Åkesson and Jonsson, 1997; Åkesson and Paulsson, 1997; Wells and Digenis, 1988; Sitarek and Kilanowicz, 2006). Main NMP metabolite is 5-hydroxy-N-methylpyrrolidone, which was detected in rats administered ^{14}C -NMP intravenously (Payan et al., 2002).

This study was aimed at evaluating the relationship between the dose level of NMP given orally and its effect on fertility in female rats and early development of their progeny.

MATERIAL AND METHODS

The present study was performed in conformity with current Polish and EU regulations (Official Gazette No. 11 of August 21, 1997 item 727, Act on Protection of Animals, Section 9: Experimental Procedures with the Use of Animals). The research team has obtained the consent to proceed with the tests of animals (Document No. Ł/BD/165, of March 24, 2003) from the Biomedical Ethics Committee, Lodz, Poland.

Chemical

NMP (CAS 872-50-4) of purity > 98% was obtained from Sigma (SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany).

Animals

Adult virgin female and male rats of the Wistar strain were obtained from the Nofer Institute's own breeding colony. Before the testing, the animals had been subjected to a 5-day adaptation. The animals were housed in plastic cages containing sterile paddy husk as bedding material with controlled temperature of $22 \pm 2^\circ\text{C}$, and light/dark cycle 12:12 hr (light on at 6:00 AM), and relative humidity of 45 to 55%. They received commercial pellet diet (Fodder Factory, Motycz, Poland) and water ad libitum.

Assessment of Females Fertility and Development Their Progeny

Exposure and observation of mothers. The parental cohort used to assess females in fertility consisted of 100 twelve-week-old virgin females and 50 fourteen-week-old control males. Number of females in groups: control 24 females; 150 mg/kg group – 26; 450 mg/kg group – 28 and 1000 mg/kg group – 22 females. The females were exposed by gavage to NMP 5 day/week at daily doses 150, 450, or 1000 mg/kg (4, 12, and 26% of oral LD_{50}). The controls received by gavage 5 days/week equivalent quantities of tap water. Each time, the animal from control and exposure groups received 0.5 ml per 100 gm b.w. The exposure period comprised about 9 weeks (2 weeks before mating and 1 week of mating,

3 weeks of gestation, and 3 weeks of lactation). It is some modification of one-generation reproduction toxicity study (OECD 415). Body weight and daily food and water consumption of females were measured two times before mating, and on 0, 6, 13, and 20 days of gestation, and two times during lactation.

Mating, conception control, and assessment of the offspring. After a 2-week exposure to NMP, females from each of four exposure groups were mated overnight with no exposed males (two females to one male). The morning when copulation plugs or spermatozoa in vaginal smears appeared was regarded as day 0 of gestation; the female was weighted and transferred back to its home cage.

On the first postnatal day (PND 1), the live and dead pups were counted, weighted, and their gender was determined. On day 4 after birth, the litters were culled to eight animals each and balanced for gender (four females and four males) to the extent possible. From birth (PND 1) to weaning (PND 21), the offspring was assessed for the general appearance, litter weight, mean pup weight, and mortality.

Sacrifice and histopathological examinations of mothers. Females from group 0, 150, and 450 mg/kg were necropsied after 3 weeks of lactation and from group 1000 mg/kg/day with no delivery—at 25th day post mating.

The animals were decapitated under light ether anesthesia, and integral indices of toxicity, including body weight on the day of dissection, hematocrit, macroscopic assay of the internal organs, absolute and relative weight of the internal organ (liver, kidneys, spleen, brain, ovaries, adrenals, thyroid, and pituitary) were determined.

Pathological examinations were performed in 15 females treated with NMP at 1000 mg/kg b.w. and in animals of groups exposed to NMP at 150 and 450 mg/kg and the control group (20–24 females in each group). The following organs were selected for histopathological examinations: ovaries, uterus, adrenal gland, lung, liver, kidneys, spleen, and brain. The organs were fixed with 10% formalin in phosphate buffer. The tissues were prepared using routine paraffin technique. The 4- to 6- μm paraffin sections were stained with hematoxylin and eosin.

Statistical Analysis

One-way analysis of variance and Dunnett's test were used in the case of variance homogeneity; Kruskal–Wallis analysis of variance was followed by nonparametric test in the case of heterogeneity. Frequency data were analyzed with Fisher's exact probability test (Zar, 1974). The effect on food and water consumption and body weight gain was evaluated by repeated measures analysis of variance (Fisher and Belle, 1993). In all statistical analyses, the difference between the compared variables was assumed to be statistically significant at $p < 0.05$.

RESULTS

Assessment of Female Fertility

Treatment with NMP did not affect the survival of female rats, although some deaths were noted in the

Table 1
Body Weight (gm), Daily Food (gm/dam), and Water (ml/dam) Consumptions During Exposure of Female Rats Receiving N-Methyl-2-Pyrrolidone Orally*

Parameters	Daily dose of NMP (mg/kg)	No. of dams per group	Weeks before mating		Days of gestation				Days of lactation ^c
			First	Second	0	6	13	20	
Body weight (gm)	0	22	203.5 ± 10.7 ^a	211.0 ± 13.2	215.1 ± 16.2 ^a	254.2 ± 15.0	284.5 ± 18.1	321.3 ± 29.0	253.6 ± 11.8
	150	24	198.4 ± 10.2	204.2 ± 11.7	206.5 ± 10.8	223.7 ± 8.7 ^b	243.9 ± 10.5 ^b	299.6 ± 11.1 ^b	254.7 ± 12.1
	450	20	200.0 ± 11.0	201.9 ± 13.8	204.0 ± 12.6	224.0 ± 14.6 ^b	243.0 ± 16.5 ^b	284.3 ± 40.4 ^b	251.3 ± 21.0
	1000	15	206.1 ± 16.8	208.7 ± 17.4	216.4 ± 16.8	245.7 ± 19.9	253.2 ± 17.6 ^b	259.7 ± 17.9 ^b	–
Food consumptions (gm/dam)	0	22	17.8 ± 0.9	19.6 ± 1.4	18.8 ± 1.9	20.8 ± 2.7	22.7 ± 2.8	20.4 ± 3.4	62.4 ± 16.4
	150	24	16.9 ± 1.7	16.8 ± 2.0	17.6 ± 2.2	19.3 ± 1.8	20.5 ± 2.5	20.2 ± 3.1	60.2 ± 23.1
	450	20	15.9 ± 1.7	15.8 ± 2.0	16.3 ± 1.8	18.8 ± 1.5	20.2 ± 2.3	21.5 ± 2.1	55.1 ± 14.1
	1000	15	15.7 ± 2.6	18.2 ± 2.1	14.9 ± 1.6 ^b	19.2 ± 2.0	15.8 ± 2.8 ^b	14.1 ± 2.7 ^b	–
Water consumptions (ml/dam)	0	22	20.2 ± 2.9	25.0 ± 4.0	32.8 ± 2.2	34.0 ± 4.6	34.9 ± 4.9	38.8 ± 5.3	103.8 ± 25.3
	150	24	23.1 ± 3.5	30.0 ± 2.2	31.8 ± 5.9	33.0 ± 3.1	34.1 ± 4.4	38.3 ± 5.4	96.9 ± 19.4
	450	20	22.8 ± 4.9	26.4 ± 5.7	32.2 ± 4.7	33.4 ± 4.7	33.9 ± 4.5	39.6 ± 3.9	89.9 ± 23.1
	1000	15	18.8 ± 5.3 ^b	24.7 ± 5.3	28.8 ± 4.3 ^b	32.1 ± 5.1	34.9 ± 5.9	31.6 ± 6.1 ^b	–

*Observations concern only pregnant females.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

^cObservations of mothers from group 1000 mg/kg have not been conducted because their offspring survived only until the four PND.

highest dose group. One female died in the 30th day and one in the 32nd day of experiment. Pneumonia was diagnosed by histopathological examination; both females were nonpregnant. Integral toxicity indices (body weight, body weight gain, food and water consumption, absolute and relative weight of internal organs, and results of pathomorphological examination) indicate that NMP administered orally at doses 450 and 1000 mg/kg/day is toxic for female rats. NMP at these doses disturbs fertility of dams, viability of their progeny, and developmental toxicity of offspring during lactation period. At the lowest dose—150 mg/kg, slightly toxic for dams—substance does not disturb their fertility but affects their offspring. Body weight of females before mating was similar in all groups. However, the trend of weight gain during pregnancy differed significantly in all groups of females exposed to NMP compared to weight gain of control females (Table 1). Daily food intake by the females of the 1000 mg/kg NMP group was significantly lower than in controls at 0, 13, and 20 days of gestation, and daily water intake by the females was significantly lower at 0 and 20 days of gestation (Table 1).

The numbers of live pups in the control group and groups exposed to NMP at 150 and 450 mg/kg were similar. The number of live pups in the group exposed to the highest dose was significantly lower and number of dead pups in litters was significantly greater than in controls. Survival of the pups from all exposed groups during the 3 weeks after birth was significantly lower than the young control animals (Table 2).

Fertility index (percentage of pregnant females in mating females group) did not differ significantly in the control and the group exposed to NMP at 150 mg/kg/day. We found a significantly lower value of this index in the groups of females exposed to the higher doses. Of

the 28 females from the group exposed to NMP at 450 mg/kg/day and mated with males, 71.4% of the females were pregnant. In the group exposed to NMP at dose 1000 mg/kg/day, of the 22 mated females, 15 were pregnant and of these pregnant females only 7 delivered pups (Table 2). All male rats were fertile because each of them inseminated at least one of two females mated with him.

Viability index (percentage of pups born alive that survived to 4 days) indicated that the offspring of all exposed female groups were characterized by significantly lower survival of pups in the first 4 days after birth compared to control. Similarly, the evaluation of lactation performance and survival of the offspring during the 3 weeks after birth, expressed as a lactation index (percentage of pups alive at 4 days that survived to 21 days), had significantly lower values compared to the control group (Table 2).

Body weight of pups (both genders) fed by females exposed to NMP at 150 or 450 mg/kg was significantly lower than the weight of the control pups starting from PND 4 until the end of the observation of the offspring, that is, to PND 21. The offspring exposed prenatally and postnatally to NMP at 150 mg/kg had lower body weight at PND 4 and 14 (females) and PND 4 (males) than the control offspring at the same time (Table 3).

Results of examinations of the females exposed to the highest (1000 mg/kg) dose of NMP require separate discussion. In that group, of the 22 inseminated females, 15 were pregnant, and pups were delivered by only 7 of them. The number of newborns in that group was only three live-born and five stillborn. All pups of this group died within the first 4 days after birth, which prevented further observation of the offspring (Table 1). To determine whether other eight inseminated females (in addition to the seven females that gave offspring) were

Table 2
Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5 ^a	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82 ^b
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1 ^b
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4 ^b	68.2 ^b
Viability	94.0	86.4 ^b	71.6 ^b	0
Lactation	96.1	78.2 ^b	43.4 ^b	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.

^aMean ± SD.

^bSignificantly different ($p < 0.05$) from control value.

F, female; M, male; GD, gestation day.

Index of fertility—percentage of pregnant females in mating females group.

Index of viability—percentage of pups born alive that survived to 4 days.

Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

Table 3
Body Weight of Offspring Control and Exposed Prenatally and Postnatally to *N*-Methyl-2-Pyrrolidone

Groups of NMP (mg/kg)	Days of live				
	1	4	7	14	21
Females					
0 (<i>n</i> = 22)	5.55 ± 0.51 ^a	9.79 ± 1.42	12.14 ± 1.05	23.35 ± 1.95	32.17 ± 2.85
150 (<i>n</i> = 24)	5.47 ± 0.48	7.35 ± 1.20 ^b	10.97 ± 2.08	21.57 ± 4.10 ^b	32.31 ± 3.17
450 (<i>n</i> = 20)	4.93 ± 0.36	6.07 ± 0.83 ^b	8.01 ± 1.63 ^b	17.81 ± 3.77 ^b	27.46 ± 4.66 ^b
Males					
0 (<i>n</i> = 22)	6.03 ± 0.78	11.05 ± 1.13	13.13 ± 1.63	24.28 ± 1.88	33.79 ± 2.77
150 (<i>n</i> = 24)	5.73 ± 0.49	7.70 ± 1.29 ^b	11.55 ± 1.85	22.71 ± 3.55	32.83 ± 4.83
450 (<i>n</i> = 20)	5.13 ± 0.39	6.34 ± 1.02 ^b	8.57 ± 1.85 ^b	19.73 ± 3.07 ^b	29.08 ± 2.50 ^b

n, number of letters in the group.

^aMean ± SD.

^bSignificantly different (*p* < 0.05) from control values.

pregnant, they were necropsied 25 days after insemination. Early resorptions were found in eight of the necropsied females, indicating a substantial intrauterine mortality of embryos. Thus, of the total of 22 inseminated females exposed to the highest NMP dose, 15 were pregnant, representing 68% of the mated females. Microscopically endometritis and foci of resorption were noted in uterus of eight females sacrificed on 25th day after insemination. In four of these females, the number of corpora lutea in the ovaries was less than in controls.

Table 4 shows the absolute and relative organ weight of females at the time of necropsy, that is, after the end of the lactation period and after the pups had been separated from their mothers. The mean relative thyroid weight of females of the group exposed to NMP at 450 mg/kg/day was significantly higher than in the control. Absolute and relative weights of other organs in the exposed females did not differ significantly from the weights of the respective organs of control females. Hematocrit value dropped with increasing doses of NMP with which the females were treated, and the difference was statistically significant (Table 4). Weights of the internal organs of the 1000 mg/kg group females necropsied 25 days after insemination were not analyzed. Their exposure period was shorter than in remaining groups, only 2 weeks before mating, 3 weeks during gestation, and 4 days during lactation. All pups of this group died within the first 4 days after birth and exposure of dams was finished.

In pathomorphological examination, there were no perceptible lesions in the lungs, liver, kidneys, spleen, brain, adrenal gland of the control females, and those exposed to NMP at 150 and 450 mg/kg necropsied after lactation. Uteri of those female rats had a shape characteristic of a female with a history of pregnancy. Numerous yellow bodies and Graafian follicles at various stages of maturity were visible in the ovaries. The microscopic structure of the lungs, liver, kidneys, spleen, brain, adrenal glands of females exposed to NMP at 1000 mg/kg, and necropsied on the 25th day after insemination was normal. The ovaries differed in the number of corpora lutea in comparison with control and those females exposed to low and median dose of NMP. In the uterine mucosa and myometrium of the females of the 1000 mg/kg group, there

were visible infiltrations of mononuclear cells and granulocytes and early resorptions.

DISCUSSION

NMP is a substance that is easily absorbed into the body as a result of oral intake, inhalation, or dermal exposure (Bartek et al., 1972; Midgley et al., 1992; Ravn-Jensen et al., 1992; Ursin et al., 1995; Payan et al., 2003). In conditions of acute exposure, NMP has no significant toxic effects except for irritation. Many experimental studies indicate that NMP at doses or concentrations nontoxic to pregnant animals shows an embryotoxic, fetotoxic, or teratogenic activity. In rats exposed orally at 332 and 997 mg/kg during organogenesis, NMP was embryotoxic and teratogenic, although those doses were not toxic to the mothers (Solomon et al., 1996). NMP induced skeletal and visceral malformations in the fetuses prenatally exposed orally at doses toxic to the mothers (500 and 750 mg/kg), while at dose 250 mg/kg, nontoxic to pregnant females, NMP was fetotoxic. At higher doses, 500 and 750 mg/kg, NMP caused imperforate anus, absence of tail, malformations of the great vessels, and ossification abnormalities. At 125 mg/kg, there was no observed maternal or developmental toxic effect (Saillenfait et al., 2002).

Inhalation exposure of female rats to NMP at 100 to 360 mg/m³ since 6 till 15 days of gestation did not disturb the prenatal development in the offspring (Lee et al., 1987), while the exposure since 4 till 20 days of gestation at 660 mg/m³, in spite of no toxic effects in the mothers, showed embryo- and fetotoxic impact (Jakobsen and Hass, 1990).

Prenatal development disorders manifested by delayed ossification were observed in the offspring of female rats exposed by inhalation from day 4 to 20 of gestation to NMP at 680 mg/m³ (Hass et al., 1995). The study also revealed increased preimplantation losses, but not significantly different number of implants and live fetuses per litter from control and exposed females.

Delayed postnatal development was observed in the offspring of females exposed to NMP at 622 mg/m³ from 7 to 20 days of gestation (Hass et al., 1994). In both studies cited above, NMP at 680 and 622 mg/m³ was nontoxic to pregnant females, while it had a negative impact on their

Table 4
Absolute and Relative (per 100 gm Body Weight) Organ Weight of Female Rats Receiving Daily Oral Dose of NMP Before Mating, During Gestation and Lactation*

Organ	Parameters	Daily dose of NMP (mg/kg)		
		0 <i>n</i> = 22	150 <i>n</i> = 24	450 <i>n</i> = 20
Ovaries	Weight (mg)	81.3 ± 10.58 ^a	88.0 ± 20.01	89.0 ± 17.02
	Relative weight (mg%)	31.9 ± 3.78	35.49 ± 8.08	33.6 ± 10.25
Liver	Weight (gm)	10.87 ± 1.28	11.0 ± 1.56	10.89 ± 1.47
	Relative weight (gm%)	4.33 ± 0.41	4.43 ± 0.56	4.32 ± 0.48
Spleen	Weight (gm)	0.54 ± 0.07	0.58 ± 0.11	0.57 ± 0.09
	Relative weight (gm%)	0.21 ± 0.02	0.23 ± 0.05	0.23 ± 0.04
Brain	Weight (gm)	1.56 ± 0.14	1.60 ± 0.07	1.56 ± 0.10
	Relative weight (gm%)	0.62 ± 0.07	0.65 ± 0.06	0.62 ± 0.06
Kidneys	Weight (gm)	1.53 ± 0.13	1.57 ± 0.14	1.57 ± 0.11
	Relative weight (gm%)	0.61 ± 0.04	0.63 ± 0.04	0.62 ± 0.05
Adrenals	Weight (mg)	58.8 ± 6.27	60.0 ± 11.0	57.0 ± 10.0
	Relative weight (mg%)	23.5 ± 2.99	24.2 ± 3.85	22.45 ± 3.92
Thyroid	Weight (mg)	12.6 ± 2.92	15.0 ± 4.0	16.0 ± 4.0
	Relative weight (mg%)	5.01 ± 0.99	5.99 ± 1.63	6.32 ± 1.99 ^b
Pituitary	Weight (mg)	12.0 ± 2.02	12.0 ± 4.0	12.0 ± 4.0
	Relative weight (mg%)	4.61 ± 0.91	4.86 ± 1.48	4.57 ± 1.36
Body weight	(gm)	253.6 ± 11.8	254.7 ± 12.7	251.3 ± 21.0
Hematocrit	(%)	48.5 ± 2.0	46.7 ± 3.1 ^b	46.1 ± 2.6 ^b

Females were necropsied after 3 weeks of lactation.

n, the number of rats in the group.

*All pups of this group died within the first 4 days after birth, which prevented further observation of the mothers and offspring.

^aMean ± SD.

^bSignificantly different (*p* < 0.05) from the control group.

offspring, even though the animal exposure time was relatively short, of the order of several (more than 10) days.

Maternal toxicity and evident fetotoxicity were observed in rats exposed 6 hr/day on 6 through 20 days of gestation to NMP at 492 mg/m³ but in lower concentrations 123 and 246 mg/m³ this chemical did not produced maternal and developmental toxicity (Saillenfait et al., 2003).

In the present study, NMP has been found to exert a significant effect on female fertility. NMP impaired female fertility when the substance was administered at 450 and 1000 mg/kg, while general toxicity of this substance (reduction of body weight and daily intake of food and water during gestation) was observed in females from the exposed groups. However, a significant reduction of offspring survival and adverse effects on the process of lactation was found even when the dose was much smaller, approximately 4% of the LD₅₀ oral for the rat. At 150 mg/kg/day, NMP revealed no significant maternal toxicity, while reduced survival of the offspring was noted during the postnatal period. Threefold higher oral doses to females caused only slight overall maternal toxicity (increased relative thyroid weight and lower hematocrit value compared to nonexposed females) but resulted in delayed physical development of the offspring (both genders). NMP administered at 1000 mg/kg/day (26% of LD₅₀) was toxic to females, especially pregnant females, and significantly reduced their fertility and led to a significant increase in mortality of the offspring.

Our results indicate that the offspring of females of all exposed groups was characterized by significantly lower survival of the pups in the first 4 days after birth com-

pared to control. This is evidenced by the low viability index (percentage of pups born alive that survived to 4 days). Similarly, the evaluation of lactation performance and survival of the pups during the 3 weeks after birth, expressed as the lactation index (percentage of pups alive at 4 days that survived to 21 days), showed significantly lower values than the corresponding values for the control group. In summary, the results of our study confirm the fact that although females were exposed to NMP at doses that were toxic (450 and 1000 mg/kg/day), this dose caused a decreased fertility of dams and, in addition, an increased intrauterine mortality. NMP at dose 450 mg/kg can decrease viability and physical development of the offspring in early postnatal period. This substance in slightly toxic dose of 150 mg/kg/day for mothers can be toxic for offspring and decrease viability of young rats in early postnatal period.

Saillenfait et al. (2007) has reported that the original NMP is more responsible for the reproductive toxicity than its two major metabolites, 5-hydroxy-N-methylpyrrolidone and 2-hydroxy-N-methylsuccinimide, which do not show an embryotoxic or fetotoxic effect, while the third, N-methylsuccinimide, is embryotoxic and teratogenic but only in the presence of maternal toxicity. Note, however, that the N-methylsuccinimide was much less potent than the NMP.

CONCLUSION

Intragastric exposure of female rats to NMP before pregnancy, during gestation, causes significant impairment in female fertility and intrauterine mortality rates.

At lower doses, toxic or slightly toxic to the mothers, NMP causes decrease in viability and physical development of progeny in the postnatal period when exposure was before pregnancy, during prenatal and early postnatal period.

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