EFFECTS ON GROWTH OF RAT OFFSPRING BORN FROM DAMS TREATED SUBCUTANEOUSLY WITH A SURFACTANT, POLYOXYETHYLENE (10) NONYLPHENYL ETHER (NP-10), DURING LACTATIONAL PERIOD

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ABSTRACT — A surfactant NP-10 was administered subcutaneously to Jcl:Wistar female rats at dose levels of 5, 20 and 80 mg/kg/day from date of birth to day 21 after birth of F1 offspring to assess its effects on the growth, behavior and functions of the offspring.

For F0 dams, scab formation and loss of hair at the test substance administration site were observed in all treatment groups and induration of the skin at the test substance administration site in the 20 and 80 mg/kg groups in general condition and necropsy findings at the end of the dosing period. In necropsy findings, in addition to these changes, hemorrhage and whitish change of the subcutis at the test substance administration site were seen in all treatment groups, adhesion to the somatic muscles and granulation of the subcutis at the test substance administration site in the 20 and 80 mg/kg groups, and swelling of the spleen and adrenals in the 80 mg/kg group. Reduction or a tendency for reduction in food consumption was also detected from the initial day of dosing (day 0) to day 17 after birth F1 offspring in the 80 mg/kg group. Body weight or the findings on the day after birth and day of weaning failed to reveal any evidence of an effect that could be ascribed to the test substance.

In F1 born offspring, a decrease or tendency for decrease in body weight was observed from day 7 after birth in both sexes and for females during the gestation period in the 80 mg/kg group. However, body weight gains based on the weights at 4 weeks after birth or on day 0 of gestation in the 80 mg/kg group failed to reveal any difference from that in the control group. The observation on the day of birth and during the period of lactation, physical development test, reflex test, general condition, open-field test, water-maze test, reproductive ability test, observations at cesarean section, necropsy findings, organ weights, histopathological findings of females or males that did not achieve successful gestation, skeletal examination, and the observations at cesarean section and external examination of F2 fetuses failed to reveal any evidence that could be ascribed to the test substance.

These results indicate that NP-10 had no effect on the behavior or functions of the offspring, although it affected the growth of the offspring born, under the conditions of this study. The non-effective dose level is considered to be 20 mg/kg for general toxicity of the dams and for their offspring.

KEY WORDS: Polyoxyethylene(10)nonylphenyl ether, Subcutaneous, Lactation, Rats
INTRODUCTION

A surfactant, polyoxyethylene(10)nonylphenyl ether (NP-10), with the nonproprietary name nonoxynole, has a spermicidal action (Iwahara et al., 1965; Tanaka and Tangezaka, 1961), and is an active contraceptive ingredient.

There are several reports regarding the general toxicity of NP-10 or its derivative in experimental animals. Acute toxicity was very weak, since LD50 was 2 or 3 g/kg in rats, mice or rabbits by oral administration (Kitagawa et al., 1981; Olson et al., 1962; Okahara et al., 1963; Tanaka and Tangezaka, 1961). Subchronic or chronic toxicity was also weak by oral or intravaginal administration in rats or rabbits (Kitagawa et al., 1981; Olson et al., 1962; Okahara et al., 1963; Smyth and Calandra, 1969). Regarding the reproduction study, Abrutyn et al. (1982) reported that N-9 (Delfen contraceptive cream), which is a derivative of NP-10, had no teratogenicity in rats by intravaginal administration during the fetal organogenesis period. On the other hand, Meyer et al. (1988) reported that fetal body weight was decreased in rats by oral administration of N-9 throughout pregnancy or during the fetal organogenesis period. However, there is no information concerning effects on offspring born from dams treated with NP-10 or its derivative.

To access the effects of NP-10, it was administered subcutaneously to rat dams during the lactation period, from date of birth to day 21 after birth of Fi offspring, and the development of the offspring was examined. The administration period was decided by referring to the reproductive and developmental toxicity study guidelines for toxicity of drugs in Japan.

MATERIALS AND METHODS

Test substance

NP-10 (Lot No. FF-5031) is a sticky, pale-yellowish liquid which is easily soluble in water and almost insoluble in petroleum ether. The pH value in this case was 6.4. It was stored at room temperature since NP-10 is stable for one year at room temperature.

Animals and housing conditions

Ten-week-old female and 11-week-old male Jcl: Wistar rats (specific pathogen-free, Clea Japan, Inc.) were purchased for use in this study. Animals were quarantined and acclimatized for 10 days. Only healthy animals showing favorable body weight gain and good general condition were used for the study. To obtain pregnant animals, females were placed into the male’s cage in the evening and two females were housed overnight with each male. Females were examined the next morning for the presence of vaginal plugs or sperm in vaginal smears in order to determine that copulation had occurred. The day on which evidence of copulation was found was designated day 0 of gestation. To minimize the differences in body weight among the groups, we allocated the pregnant animals into groups of 24 to confirm pregnancy. At the start of mating, age and body weight were 11-week-old and 202.8-231.0 g for the females and 12-week-old and 323.6-376.8 g for the males.

The animals were housed in an animal room kept at a temperature set at 23 ± 2°C, with relative humidity 55 ± 10% and a ventilation rate of 10-15 times/hr, lit for 12 hr daily (from 7:00 to 19:00). Fi dams and their litters were housed in polycarbonate cages (280 W × 440 D × 150 H mm) with nesting materials (chaff, wood chips, Chiba Animal Material Co., Ltd., Japan) from day 17 of Fi dams’ gestation until the day Fi pups were weaned, and in suspended stainless steel cages (260 W × 380 D × 180 H mm) throughout the other period (for each 2 females and for each male during the quarantine and acclimation period and mating period, for each 1 female from the day on which evidence of copulation was found till day 16 of Fi dam’s gestation, 2 or 3 animals for each sex from weaning till 10 weeks after birth, for each 1 female and 1 male during the mating period of Fi offspring, and for each 2 males and for each female after the day on which evidence of copulation was found of Fi offspring). The animals were allowed free access to pelleted diet (CRF-1, Oriental Yeast Co., Ltd., Japan), and to water from the Hita City supply via sipper tubes from an automatic waterer or in a supply bottle.

Dosing

The clinical dosing route of NP-10 is intravaginal. However, the subcutaneous route was selected by reason that a greater amount of NP-10 can be dosed by the subcutaneous route rather than the intravaginal route, and the absorption route of these routes may be similar.

Chvapil et al. (1980) reported that the detergent was detected in the milk of lactation rats and the serum of their pups within 2 hr after the intravaginal dose of radioactive N-9, and assuming the average daily volume of milk produced by lactation rats was 10 mL, 0.3% of the administered dose of the detergent was secreted in the daily production of the milk.

In the preliminary study NP-10 was dosed subcu-
Effects of NP-10 on growth of rat offspring.

taneously at dose levels of 10, 20, 40, 80, 160 and 320 mg/kg/day for 5 weeks. Suppression of body weight and deaths were observed in the groups of 160 mg/kg/day or more. Therefore 80 mg/kg/day was chosen as the high dose. Five mg/kg/day, which was about 5 times higher than the dose used in clinical application, was used as the low dose and 20 mg/kg/day as the medium dose.

NP-10 was dissolved in isotonic sodium chloride solution (Otsuka Pharmaceutical Factory, Inc.) and administered to the subcutis of the dorsal regions once a day during 9:00-11:30 with a disposable syringe. For the local irritation effect of the test substance, the administration region was changed in each dosing. The concentrations of the test substance were changed for each group and 3 mL volume per kg body weight was dosed. The same volume of the vehicle was administered to the control group.

Observations

1. F1 dams

The general condition was observed once a day from day 0 of gestation to day 22 after birth of F1 offspring and the condition of nursing from day 0 to day 22 after birth of F1 offspring. Body weight was measured on days 0, 7, 14 and 21 of gestation, date of birth, days 4, 7, 10, 14, 17 and 21 after birth of F1 offspring, and food consumption on days 1, 7, 14 and 21 of gestation, and days 1, 4, 7, 10, 14, 17 and 21 after birth delivery. The gestation period, gestation index and live birth index were investigated. The animals were euthanised by exsanguination under ether anesthesia for necropsy on day 22 after delivery.

2. F1 born offspring

The number of F1 offspring birth, the number of stillborn, the number of dead newborn, the number of live newborn and the sex ratio of live newborn were recorded and the external appearance, including the oral cavity of the live newborn, were examined on the day of birth.

During the lactation period, the general condition of the pups was examined daily, deaths were recorded, and dead offspring were necropsied as soon as possible after death. Body weight was measured on the day of birth and on days 4, 7, 10, 14, 17 and 21 after birth. On day 4 after birth, litters were culled to 8 pups (4 of each sex). For litters of 8 pups or less, all the pups were reared, and even when the number of either sex was less than 4, the total number of pups reared was eight. The pups of each litter were allocated as follows: 1 male and 1 female were necropsied on weaning day and skeletal tested; 1 male and 1 female were reflex tested, and water-maze tested before being necropsied 10 weeks after birth for organ weight measurement; 1 male and 1 female were open-field tested before being necropsied 10 weeks after birth for organ weight measurement; 1 male and 1 female were reared for 12 weeks after birth and then examined for reproductive ability. All pups alive at the time of testing were tested for physical development: pinna unfolding on day 4 after birth, growth of hair on the back on day 8, appearance of the lower incisors on day 11, quadruped walking on day 14, eye opening on day 15, testicular descent on day 21 and vagina opening on day 39. Animals which failed to show the expected physical development were continuously examined until the development change occurred. One male and 1 female of each litter in each group for the reflex test were examined for surface rights reflex and systemic pain response on day 5 after birth, negative geotaxis on day 8, pinna reflex on day 13 using an audiometer (PA-1, Nagashima Medical Equipment Co., Ltd.), and corneal reflex on day 17. One male and 1 female of each litter in each group were euthanised by exsanguination under ether anesthesia for necropsy on day 22 and treated according to the method of Staples and Schnell (Staples and Schnell, 1964) to prepare cleared skeletons stained with alizarin red S, for the examination of skeletal abnormalities, variations and the degree of ossification.

After being weaned, the general condition of the remaining 3 males and 3 females of each litter in each group was examined daily, and the number of deaths was recorded, up to 12 weeks after birth, and dead animals were necropsied as soon as possible after death. Body weight was measured once a week. During this period, 1 male and 1 female of each litter in each group were examined in an open-field test at 7 weeks after birth and 1 male and 1 female in a water-maze test at 9 weeks. In the open-field test, the animals were examined for 2 min once a day for 3 consecutive days, for latency, the number of ambulations, number of rearings, number of groomings, number of preenings and frequency of defecation and urination. The test was performed in a darkened test room, in which a circular apparatus was set up, and the whole field was lit with a 100-watt glow lamp installed 1 m above the bottom of the apparatus. The water-maze test was performed up to 3 min per test 3 or 4 times a day for 4 consecutive days using Biel's apparatus (Biel, 1940) with the water at room temperature. The animal's swimming ability (i.e. the time required to reach the goal in a straight
course on the first day, and the time required to reach
the goal and the number of errors made in a T maze
course on the second, third and fourth days) was exam-
ined.

Ten weeks after birth, 2 males and 2 females of
each litter in each group which had been used for the
reflex test, open-field test and water-maze test were
exerted by exsanguination under ether anesthesia
and necropsied. The wet weights of the brain, lung,
heart, liver, kidneys, spleen, testes, hypophysis, thyroid,
thymus, adrenals and ovaries were recorded.

Twelve weeks after birth, 1 male and 1 female of
each litter in each group were mated up to 14 days on a
1:1 basis within the group, but avoiding pairing of sib-
lings, to investigate the number of days required for
successful copulation (duration of mating), copulation
index and fertility index. Females that had copulated
successfully were weighed on days 0, 4, 8, 12, 16 and
20 of gestation. All females were euthanized by exsang-
guination under ether anesthesia on day 20 of gestation
and all males were euthanized by exsanguination under
ether anesthesia after that for necropsy. Paired females
and males that did not achieve successful pregnancy
were sacrificed and the testes, epididymis, seminal
vesicle including the coagulation gland, prostates and
hypophysis of the males, and the ovaries, uterus and
hypophysis of the females were examined histopatho-
logically.

3. F₂ fetuses

F₂ dams were sacrificed during cesarean section
on day 20 of gestation and their fetuses were examined
to determine the number of corpora lutea, number of
implantations, number of resorptions (classified as ear-
ly or late resorptions, or dead fetuses), number of live
fetuses, sex ratio of live fetuses, body weight of live
fetuses, placental weight of live fetuses, and their ex-
ternal appearances including the oral cavity.

Statistical analysis

With regard to body weight, food consumption,
the number of implantation sites, gestation period of F₀
dams, number of born offspring, number of live new-
born, body weight, body weight gain, number of ossi-
fied cervical vertebral bodies and arches and caudal
bodies and arches, organ weight, duration of mating
and number of corpora lutea of F₁ offspring and num-
ber of implantations, number of live fetuses, body
weight of live fetuses, and placental weight of live
fetuses F₂ fetuses, a one-way analysis of variance was
used to assess the significance of variance in all the
groups. When a significant difference was revealed in
this analysis, the difference between the control group
and each of the treatment groups was analyzed by
Dunnett's multiple comparison test (Dunnett, 1964).
When a significant difference was not revealed, the
characteristics were statistically analyzed by the Kruskal-
Wallis rank sum test (Kruskal and Wallis, 1952), and
statistically compared between the control group and
each of the treatment groups by Dunnett's multiple
comparison test. To analyze the gestation index, live
birth index of F₀ dams, sex ratio of live newborn, copu-
lation index and fertility index of F₁ offspring and sex
ratio of live F₂ fetuses, Fisher's exact probability test
(Fisher, 1950) was used to compare the control group
to each of the treatment groups. The rate of stillborn,
rate of external abnormalities, viability index on day 4
after birth, weaning index, rate of appearance of physi-
cal development, rate of appearance of items in the
reflex test, rate of items in the open-field test, and rate
of items in the water-maze test, rate of skeletal abnor-
malities and variations, degree of ossification of F₁
offspring and rate of pre-implant losses, rate of embryonic
and fetal deaths, rate of resorptions, rate of dead fetu-
es, and rate of external abnormalities of F₂ fetuses were
statistically analyzed by the Kruskal-Wallis rank sum
test, and statistically compared between the control
group and each of the treatment groups by Dunnett's
multiple comparison test. The rate of stillborns, rate of
external abnormalities, viability index on day 4 after
birth, weaning index, rate of appearance of physical
development and body weight from the date of birth to
the day of weaning, as well as all the characteristics of
F₂ fetuses were analyzed using the litter as a unit.

RESULTS

F₀ dams
1. General condition

Scab formation at the test substance administra-
tion site was observed in 15 animals, loss of hair at the
test substance administration site in 6 animals and red-
dish tear and nasal bleeding in 1 animal each in the 5
mg/kg group. Scab formation in every animal, loss of
hair in 15 animals and induration of the skin in 2 ani-
mal were observed at the test substance administration
site in the 20 mg/kg group. Scab formation and indura-
tion of the skin at the test substance administration site
in every animal, loss of hair in 19 animals at the test
substance administration site and nasal bleeding in 1
animal were observed in the 80 mg/kg group. In addi-
tion, scab formation at the vehicle substance adminis-
2. Body weight
Almost the same body weight changes as those in the control group were observed during the lactation period in the 5 mg/kg group. Almost the same body weight changes as those in the control group, except for a significant increase at day 21 after delivery of F1 offspring, were observed during the lactation period in the 20 mg/kg group. A tendency for decrease in body weight was seen from day 0 to day 14 after delivery in the 80 mg/kg group. (Fig. 1)

3. Food consumption
The level of food consumption in the 5 and 20 mg/kg groups was similar to that in the control group during the lactation period. A tendency for decrease from day 1 to day 7 after delivery of F1 offspring and decrease from day 10 to day 17 were observed in the 80 mg/kg group, but the level of food consumption was similar to that in the control group at day 21 after delivery.

4. Observations on the day of birth and day of weaning
No abnormalities were detected in the gestation period, gestation index or live birth index in any of the treatment groups. (Table 1)

5. Necropsy findings
Hemorrhage of the subcutis in 16 animals and whitish changes of the subcutis in 6 animals were observed at the test substance administration site in the 5 mg/kg group. Hemorrhage of the subcutis at the test substance administration site was seen in 20 animals, whitish changes of the subcutis at the test substance administration site in 17 animals, adhesion of the subcutis to the somatic muscle in 7 animals and granulation of the subcutis in 3 animals at the test substance administration site, dilatation of the renal pelvis in 3 animals and adhesion of abdominal organs in 1 animal in the 20 mg/kg group. Hemorrhage of the subcutis at the test substance administration site was shown in 18 animals, adhesion of the subcutis to the somatic muscle at the test substance administration site in 16 animals, whitish changes of the subcutis at the test substance administration site in 15 animals, granulation of the subcutis in 11 animals at the test substance administra-

Fig. 1. Mean body weight of F1 female rats dosed subcutaneously with NP-10 during lactation period.
tion site, enlargement of the spleen in 17 animals, enlargement of the adrenals in 10 animals, adhesion of abdominal organs in 8 animals, enlargement of the liver in 2 animals, and dilatation of the renal pelvis and enlargement of the kidney in 1 animal each in the 80 mg/kg group. No abnormalities were detected in the control group.

**F1 offspring**

1. **Observations on the day of birth and during the period of lactation**

   No abnormalities were detected in the number of born offspring, number of stillborn, number of live newborn, sex ratio of live newborn offspring, number of dead after birth, viability index on day 4 after birth or weaning index in any treatment groups. (Table 1)

2. **External examination**

   There were no anomalies in any groups, including the control group. (Table 1)

3. **Physical development test**

   All items of the physical development test, including pinna unfolding, growth of hair, incisor eruption, quadruped walking, eye opening, testicular descent and vaginal opening, did not reveal any abnormalities in females or males in any of the treatment groups.

**4. Reflex test**

   There were no abnormalities in the righting on surface, systemic pain response, negative geotaxia, pinna reflex or corneal reflex in females or males of any treatment groups.

**5. General condition**

   The number of deaths or deaths by cannibalism up to day 4 after birth were 4 males and 3 females in the control group, 6 males and 7 females in the 5 mg/kg group, 5 animals each in both sexes in the 20 mg/kg group, and 4 males and 7 females in the 80 mg/kg group. After day 4, 1 male died by cannibalism in the 20 mg/kg group and 1 female died in the 80 mg/kg group. During the gestation period, reddish tear was observed in 2 animals in the 20 mg/kg group and in 1 in the 80 mg/kg group. In addition, loss of hair and reddish tear in 1 animal each in the control group were

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### Table 1. Postnatal findings of F1 newborn rats born from dams dosed subcutaneously with NP-10 during lactational period.

<table>
<thead>
<tr>
<th>Exp. group (mg/kg/day)</th>
<th>Vehicle control</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dam</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of dams</td>
<td>21</td>
<td>20</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Number of dams delivering live newborn</td>
<td>21 (100)</td>
<td>20 (100)</td>
<td>22 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Duration of pregnancy (day)</td>
<td>22.0±0.22</td>
<td>21.9±0.31</td>
<td>22.0±0.21</td>
<td>21.9±0.30</td>
</tr>
<tr>
<td>Number of implantation sites</td>
<td>320 (15.2±1.55)</td>
<td>315 (15.8±2.84)</td>
<td>323 (14.7±2.80)</td>
<td>326 (15.5±2.02)</td>
</tr>
<tr>
<td><strong>Newborn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of newborn a)</td>
<td>289 (13.8±1.92)</td>
<td>290 (14.5±2.70)</td>
<td>287 (13.0±2.72)</td>
<td>296 (14.1±2.32)</td>
</tr>
<tr>
<td>Number of stillborn b)</td>
<td>12 (3.8)</td>
<td>7 (2.2)</td>
<td>6 (1.9)</td>
<td>9 (2.8)</td>
</tr>
<tr>
<td>Number of live newborn at birth c)</td>
<td>277 (13.2±2.04)</td>
<td>283 (14.2±2.68)</td>
<td>281 (12.8±2.79)</td>
<td>287 (13.7±2.82)</td>
</tr>
<tr>
<td>Sex ratio of live newborn at birth</td>
<td>(male/female)</td>
<td>142/135</td>
<td>151/132</td>
<td>148/133</td>
</tr>
<tr>
<td>Number of dead c)</td>
<td>7 (2.5)</td>
<td>13 (4.6)</td>
<td>10 (3.6)</td>
<td>11 (3.8)</td>
</tr>
<tr>
<td>Number of live newborns on day 4 c)</td>
<td>270 (97.5)</td>
<td>270 (95.4)</td>
<td>271 (96.4)</td>
<td>276 (96.2)</td>
</tr>
<tr>
<td>Number of live newborns just after culling c)</td>
<td>168 (8.0±0.00)</td>
<td>158 (7.9±0.45)</td>
<td>171 (7.8±0.75)</td>
<td>167 (8.0±0.22)</td>
</tr>
<tr>
<td>Number of dead d)</td>
<td>0</td>
<td>0</td>
<td>1 (0.6)</td>
<td>0</td>
</tr>
<tr>
<td>Number of weanings f)</td>
<td>168 (100)</td>
<td>158 (100)</td>
<td>170 (99.4)</td>
<td>167 (100)</td>
</tr>
</tbody>
</table>

| Number of newborn with external anomalies | 0 | 0 | 0 | 0 |

a) : Value in parentheses represents percentage of the number of dams.
b) : Value is mean±S.D.
c) : Value in parentheses represents mean±S.D.
d) : Value in parentheses represents percentage of the number of implantation sites.
e) : Value in parentheses represents percentage of the number of live newborn at birth.
f) : Value in parentheses represents percentage of the number of live newborn just after culling.

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seen. No abnormalities were detected in the 5 mg/kg group.

6. Body weight and body weight gain
Almost the same body weight changes as those in the control group were observed during the testing periods in the 5 and 20 mg/kg groups. A tendency for decrease in body weight was seen in females and males at day 7 after birth, and a significant decrease at 8 weeks after birth in males and at 10 weeks after birth in females was shown in the 80 mg/kg group. In this group, a significant decrease at day 0 of gestation and a tendency for decrease thereafter during the gestation period were also observed in females.

For body weight gain based on the body weight at 4 weeks after birth in females and males and on day 0 of gestation, a significant increase was seen at 8 weeks after birth in males of the 20 mg/kg group; the body weight gain in males of the 5 and 80 mg/kg groups and females in all treatment groups was similar to that in the control group. (Figs. 2 and 3)

7. Open-field test
In males, no abnormalities were detected in any item, including latency, ambulation, rearing, grooming, preening, defecation or urination in the 5 mg/kg group. A decrease in rearing on the first day of testing and an increase in preening on the second day of testing were observed in the 20 mg/kg group. An increase in latency on the first day of testing was seen in the 80 mg/kg group.

In females, no abnormalities were detected in any items in any of the treatment groups.

8. Water-maze test
Swimming ability and swimming time in the straight course, and swimming time and number of errors in the maze course did not reveal any abnormalities in females or males in any treatment groups. (Table 2)

9. Reproductive ability test
There were no abnormalities in the duration mating, copulation index or fertility index of both sexes in any treatment groups. (Table 2)

10. Observations at cesarean section
No abnormalities were observed in the number of corpora lutea in any of the treatment groups. (Table 3)

11. Necropsy findings
In the born offspring which were sacrificed or died since day 4 after birth, dilatation of the renal pelvis in 10 males, and accessory spleen and small testis in 1 male each were observed in the 5 mg/kg group, dilatation of the renal pelvis including 1 reddish region in the thymus in 18 males and reddish region in the thymus in 1 male in the 20 mg/kg group and dilatation of the renal pelvis in 13 males in the 80 mg/kg group. In addition, dilatation of the renal pelvis was seen in 17 males, including 1 nodule of the spermatic canal, accessory spleen and small testis in 1 male each, and agenesis of the testis and epididymis in 1 animal in the control group. Dilatation of the renal pelvis was observed in 6 females and reddish spot in the thymus in 1 female in the 5 mg/kg group, dilatation of the renal pelvis in 14 females, including milky white substance in the renal pelvis in 1 female and adhesion between the kidney and the liver in 1 female and reddish tear in 1 female in the 20 mg/kg group, and dilatation of the renal pelvis in 7 females, including 1 reddish tear in the 80 mg/kg group. In addition, dilatation of the renal pelvis was seen in 12 females, and loss of hair and vaginal atresia in 1 female each in the control group. In the findings of stillborn and deaths from day 0 to day 4 after birth, no findings considered to be related to death were detected in either sex of any treatment group.

12. Organ weights
No abnormalities were detected in the spleen, liver, heart, lung, thymus, kidney, testes, brain, thyroid, adrenals, ovaries or hypophysis in either sex in the 5 or 20 mg/kg groups. Decreased absolute testes weight in males and decreased absolute brain weight in both sexes and increased relative hypophysis weight in males were shown in the 80 mg/kg group.

13. Histopathological findings for females and males that did not achieve successful gestation
Three paired females and males did not achieve successful copulation or gestation in the control, 2 in the 5 mg/kg group and 3 in the 20 mg/kg group. Diffused atrophy of the seminiferous tubules in the testis and cell residue tubules of the epididymis were observed in 1 male each in the control and 5 mg/kg groups, and cystic formation of the pars intermedia of the hypophysis in 1 male in the 20 mg/kg group. No abnormalities were detected in females in either the control, 5 or 20 mg/kg group.

14. Skeletal examinations
There were no abnormalities in the males in either the control, 5 or 80 mg/kg group. Abnormal ossifica-
Fig. 2. Mean body weight of F1 male rats born from dams dosed subcutaneously with NP-10 during lactation period.

Fig. 3. Mean body weight of F1 female rats born from dams dosed subcutaneously with NP-10 during lactation period.
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Table 2. Mating and fertility findings of F1 rats born from dams dosed subcutaneously with NP-10 during lactational period.

<table>
<thead>
<tr>
<th>Mating time</th>
<th>Sex</th>
<th>Exp. group (mg/kg/day)</th>
<th>Vehicle control</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of animals mated</td>
<td>21</td>
<td>20</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>1st time</td>
<td>Male</td>
<td>Number of animals that copulated a)</td>
<td>20 (95.2)</td>
<td>20 (100)</td>
<td>21 (95.5)</td>
<td>21 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of animals that produced pregnant females b)</td>
<td>18 (90.0)</td>
<td>18 (90.0)</td>
<td>19 (90.5)</td>
<td>21 (100)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Number of animals mated</td>
<td>21</td>
<td>20</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of animals that copulated a)</td>
<td>20 (95.2)</td>
<td>20 (100)</td>
<td>21 (95.5)</td>
<td>21 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of pregnant animals c)</td>
<td>18 (90.0)</td>
<td>18 (90.0)</td>
<td>19 (90.5)</td>
<td>21 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration of mating d)</td>
<td>2.7±1.09</td>
<td>3.2±2.78</td>
<td>2.3±1.11</td>
<td>2.7±1.10</td>
</tr>
<tr>
<td>2nd time</td>
<td>Male</td>
<td>Number of animals mated</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of animals that copulated</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of animals that produced pregnant females</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Number of animals mated</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of animals that copulated</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of pregnant animals</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration of mating</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) : Value in parentheses represents percentage of the number of animals mated.
b) : Value in parentheses is (Number of animals that produced pregnant females/Number of males that copulated)×100.
c) : Value in parentheses represents percentage of the number of animals that copulated.
d) : Value represents mean±S.D. of days required for successful copulation.

Table 3. Reproductive parameters of F1 female rats born from dams dosed subcutaneously with NP-10 during lactational period.

<table>
<thead>
<tr>
<th>Exp. group (mg/kg/day)</th>
<th>Vehicle control</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dams</td>
<td>18</td>
<td>18</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Number of corpora lutea a)</td>
<td>289 (16.1±1.55)</td>
<td>275 (15.3±1.93)</td>
<td>306 (16.1±1.29)</td>
<td>325 (15.5±1.40)</td>
</tr>
<tr>
<td>Number of implantations a)</td>
<td>274 (15.2±1.63)</td>
<td>259 (14.4±2.75)</td>
<td>284 (14.9±2.80)</td>
<td>314 (15.0±1.40)</td>
</tr>
<tr>
<td>Number of pre-implant losses b)</td>
<td>15 (5.2)</td>
<td>16 (5.8)</td>
<td>22 (7.2)</td>
<td>11 (3.4)</td>
</tr>
<tr>
<td>Number of resorptions c)</td>
<td>22 (8.0)</td>
<td>15 (5.8)</td>
<td>24 (8.5)</td>
<td>30 (9.6)</td>
</tr>
<tr>
<td>Early resorptions c)</td>
<td>20 (7.3)</td>
<td>13 (5.0)</td>
<td>24 (8.5)</td>
<td>28 (8.9)</td>
</tr>
<tr>
<td>Late resorptions c)</td>
<td>1 (0.4)</td>
<td>2 (0.8)</td>
<td>0 (0.0)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Dead fetuses c)</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Number of live fetuses a)</td>
<td>252 (14.0±2.00)</td>
<td>244 (13.6±2.83)</td>
<td>260 (13.7±2.81)</td>
<td>284 (13.5±2.18)</td>
</tr>
<tr>
<td>Male/Female (sex ratio)</td>
<td>114/138 (0.83)</td>
<td>127/117 (1.09)</td>
<td>127/133 (0.95)</td>
<td>150/134 (1.12)</td>
</tr>
<tr>
<td>Body weights d) (g)</td>
<td>3.14±0.17</td>
<td>3.15±0.14</td>
<td>3.23±0.21</td>
<td>3.19±0.17</td>
</tr>
<tr>
<td>Male</td>
<td>2.98±0.13</td>
<td>2.97±0.18</td>
<td>3.04±0.17</td>
<td>3.04±0.19</td>
</tr>
<tr>
<td>Female</td>
<td>0.42±0.04</td>
<td>0.43±0.04</td>
<td>0.45±0.07</td>
<td>0.44±0.06</td>
</tr>
<tr>
<td>Placental weights d) (g)</td>
<td>0.40±0.03</td>
<td>0.40±0.03</td>
<td>0.43±0.10</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td>Male</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a) : Value in parentheses represents mean±S.D. per dam.
b) : Value in parentheses represents percentage of the number of corpora lutea.
c) : Value in parentheses represents percentage of the number of implantations.
d) : Value is mean±S.D.
e) : Value in parentheses represents percentage of the number of fetuses examined.

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tion of the caudal vertebra was seen in 1 animal in the 20 mg/kg group. As variations, cervical rib was observed in 4 animals and lumbar rib in 1 animal in the control group, cervical rib in 4 animals and lumbar rib in 2 animals in the 5 mg/kg group, cervical rib in 4 animals and lumbar rib in 3 animals in the 20 mg/kg group and cervical rib in 6 animals and separation of the thoracic vertebral bodies in 1 animal in the 80 mg/kg group.

There were no abnormalities in the females in any group including the control group. As variations, cervical rib was observed in 4 animals and lumbar rib in 3 animals in the control group, cervical rib in 4 animals, cervical rib and lumbar rib in 1 animal and lumbar rib in 1 animal in the 5 mg/kg group, cervical rib in 6 animals in the 20 mg/kg group and cervical rib in 3 animals in the 80 mg/kg group.

The frequencies of these findings were not significantly different from those in the control group. (Table 4)

F1 fetuses
1. Observations at cesarean section

No abnormalities were detected in the rate of pre-implant losses, number of implantations, rate of embryonic and fetal death, rate of resorptions, rate of dead fetuses, number of live fetuses, sex ratio of live fetuses, body weight of live fetuses or placental weight of live fetuses in any of the treatment groups. (Table 3)

2. External examination

There were no abnormalities in any of the treatment groups. One fetus with cleft palate and aplasia of the mandible was seen in the control group. (Table 3)

**DISCUSSION**

NP-10 was administered subcutaneously to F1: Wistar female rats at dose levels of 5, 20 and 80 mg/kg/day from date of birth to day 21 after birth of F1 offspring to assess its effects on their growth, behavior and functions.

In the general condition, scab formation and loss of hair at the test substance administration site were observed in F0 dams in all treatment groups. Scab formation was seen in all animals in the 20 and 80 mg/kg groups. In these groups, induration of the skin at the test substance administration site was also observed, and this change was seen in all animals in the 80 mg/kg group. In addition to these changes, in the necropsy findings, hemorrhage and whitish changes of the subcutis at the test substance administration site were observed in every treatment group, and adhesion of the subcutis to the somatic muscle and granulation at the test substance administration site in the 20 and 80 mg/kg groups. These changes were considered to be structure lesions resulting from the surfactant effects of the test substance. However, no other changes or abnormalities were seen in the lactation condition in any of the treatment groups. In body weight, almost the same changes as those in the control group were observed in the 5 and 20 mg/kg groups, but a tendency for decrease was seen from day 0 to day 14 after delivery of F1 offspring in the 80 mg/kg group. Since the body weight on day of delivery, meaning the body weight just before dosing in this group, was low, this

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**Table 4.** Skeletal findings of F1 rats born from dams dosed subcutaneously with NP-10 during lactational period.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Exp. group (mg/kg/day)</th>
<th>Vehicle control</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of animals examined</td>
<td>19</td>
<td>21</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Number of animals with anomalies a)</td>
<td>0</td>
<td>0</td>
<td>1 (3.7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Abnormal ossification of caudal vertebra a)</td>
<td>0</td>
<td>0</td>
<td>1 (3.7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Number of animals with variations a)</td>
<td>5 (26.3)</td>
<td>6 (28.6)</td>
<td>7 (25.9)</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td></td>
<td>Cervical rib a)</td>
<td>4 (21.1)</td>
<td>4 (19.0)</td>
<td>4 (14.8)</td>
<td>6 (26.1)</td>
</tr>
<tr>
<td></td>
<td>Separation of thoracic vertebral body a)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td></td>
<td>Lumbar rib a)</td>
<td>1 (5.3)</td>
<td>2 (9.5)</td>
<td>3 (11.1)</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>Number of animals examined</td>
<td>23</td>
<td>17</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Number of animals with anomalies</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Number of animals with variations a)</td>
<td>7 (30.4)</td>
<td>6 (35.3)</td>
<td>6 (35.3)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td></td>
<td>Cervical rib a)</td>
<td>4 (17.4)</td>
<td>5 (29.4)</td>
<td>6 (35.3)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td></td>
<td>Lumbar rib a)</td>
<td>3 (13.0)</td>
<td>2 (11.8)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a) : Value in parentheses represents percentage of the number of animals examined.

---
change was considered to be an accompanying change and not considered to be related to the treatment.

Although the level of food consumption in the 5 and 20 mg/kg groups was similar to that in the control group, an effect was observed from just after start of dosing to just before the end of dosing in the 80 mg/kg group, i.e. a significant decrease in food consumption from day 10 to day 17 after delivery of F1 offspring. Almost the same level of food consumption as in the control group was observed on day 21 after delivery of F1 offspring in this group, indicating recovery. In the necropsy findings, in addition to the changes at the test substance administration site or of the subcutis at the test substance administration site mentioned above, enlargement of the liver, spleen and adrenals and adhesion of abdominal organs were observed in the 80 mg/kg group and adhesion of abdominal organs in the 20 mg/kg group. The changes in the spleen and adrenals were considered to be due to stress from the surfactant effect of the test substance. In the observations on the day of birth and the day of weaning, the live birth index failed to reveal any abnormalities in any groups including the control group.

The body weight changes in the F1 born offspring in the 5 and 20 mg/kg groups were similar to those in the control group. A tendency for decrease or decrease in body weight was seen in females and males later than day 7 after birth, including the gestation period for females in the 80 mg/kg group. However, for body weight gain based on the body weight 4 weeks after birth in females and males or on day 0 of gestation, similar changes were seen, and the offspring was considered to develop favorably after weaning in this group. In the open-field test, the females showed no abnormalities in any items in any treatment groups. In males, a decrease in rearing was observed on the first day of testing, an increase in preening on the second day of testing in the 20 mg/kg group, and an increase in latency on the first day of testing in the 80 mg/kg group. These changes were not considered to be significant, since both changes in the 20 mg/kg group were transient and were not observed in any other treatment group, the change in the 80 mg/kg group was transient, and no changes in other items were detected. In the necropsy findings, dilatation of the renal pelvis was observed in both sexes of all treatment groups. This change was considered to be incidental, since the frequency of this change was not dosage-related and the frequency of this change was similar to that seen in the control group. No other changes attributable to the test substance were detected in the findings. Decreased absolute testes weight in males and decreased absolute brain weight in both sexes and increased relative hypophysis weight in males were shown in the 80 mg/kg group. However, these changes were not considered to be attributable to the test substance, since no changes were seen in the relative testes and brain weights or absolute hypophysis weight. In the skeletal examination, abnormal ossification of the caudal vertebra was seen in the 20 mg/kg group, and as variations, cervical rib was shown in all treatment groups, and lumbar rib in males of the 5 and 20 mg/kg groups and in females in the 5 mg/kg group. These findings were not considered to be attributable to the test substance, since their frequencies were not significantly different from those in the control group and they were not seen in the higher group or were not dosage-related. The observations on the day of birth and during the period of lactation, external examination, physical development test, reflex test, general condition, water-maze test, reproductive ability test, observations at cesarean section and histopathological findings for females and males which did not achieve successful gestation and external examination of F2 fetuses failed to reveal any abnormalities of an effect which could be ascribed to the test substance.

These results indicate that NP-10 had no effects on the behavior or function of the offspring, although it affected the growth of born offspring, under the conditions of this study. The non-effective dose level is considered to be 20 mg/kg for general toxicity of the dams and their offspring.

REFERENCES


Edinburgh.


