16) Transcriptional Assay for the Detection of Estrogenic and Anti-Estrogenic Compounds using the MELN Cells
17) New TG: KeratinoSens: An in vitro Method for Identifying the Skin Sensitisation Potential of Chemicals
18) New TG: Direct Peptide Reactivity Assay (DPRA): An In Chemico Method for Identifying the Skin Sensitisation Potential of Chemicals
19) Updated TG 431: Referencing of epiCS (previously EST1000) Skin Corrosion Test in TG 431
20) New TG: Short –Term Exposure Test (STE) for Identifying Ocular Irritants (Japan)

- Expert group met on 29-30 September 2011 and reviewed the SPSF;
- Revised SPSF and validation report submitted to WNT 24; SPSF approved;
- Comments on the validation report expected from the WNT by October 2012;
- Peer review report and draft Test Guideline expected in 2013;
- First draft TG was circulated for a first commenting round in November 2013;
- Comments received will be dealt with by the expert group in early 2014 most likely via teleconference.

21) Updated TG 421/TG 422 (Reproduction/Developmental Toxicity Screening Test)/(Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test), Enhancement with ED-relevant endpoints
22) Thyroid Scoping Document
23) New TG: Performance-Based Test Guideline on Androgen Receptor Transactivation Assays
24) Toxicity Testing on Immature/Juvenile Rodents
25) New TG on human Cell Line Activation Test (h-CLAT): an in vitro method for identifying the skin sensitisation potential of chemicals (Japan and EU)

- Validation study carried out, peer review on-going and conclusion expected by end of 2013, no further information available

26) Performance-Based Test Guideline for the establishment on human-derived hepatic system to investigate biotransformation and toxicity of compounds by evaluation of CYP450 induction competence
27) Feasibility study for a Guidance Document on Study Designs, to be used in revisions to Guidelines
28) Updated TG488, Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays

上記の中には、途中で開発が止まっているものや、遅れているものも存在する。しかしながら、今後、新たな試験法開発や、改訂を目指す場合には、これら試験法開発の状況を把握して
おくことが重要である。開発中の試験法が現在どの位置にあると認識されているか再確認でき、個別試験法開発における今後の方向性をも確認できることになる。

D. 参考資料

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研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷り

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Reproductive and developmental toxicity screening test of 3-cyanopyridine in rats

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ABSTRACT

Crl:CD(SD)rats were given 3-cyanopyridine by gavage at 0, 5, 30 or 180 mg/kg/day. Males were dosed for 42 days beginning 14 days before mating, and females for 40–53 days beginning 14 days before mating to day 3 of lactation, including throughout the mating and gestation periods. General toxicity, mainly liver damage, was observed in males at ≥30 mg/kg/day and in females at ≥5 mg/kg/day. Sertoli cell vacuolation was observed at 180 mg/kg/day, and spermatocyte damages were observed at ≥30 mg/kg/day. Effects on estrous cycles, corpora lutea and implantations, and unsuccessfully mated females, despite additional mating, were observed at 180 mg/kg/day. Delayed initiation of delivery, dystocia, and deaths or moribundities of pregnant females were observed at 180 mg/kg/day, and only two pregnant rats delivered live pups at that dose. The NOAEL for reproductive/developmental toxicity was concluded to be 30 mg/kg/day.

1. Introduction

3-Cyanopyridine (CAS No. 100-54-9) is a white to yellowish white crystalline solid with a characteristic odor [1]. In Japan, the annual production and import volume of 3-cyanopyridine was reported to be from 100 to 1000 tons in 2009 [2], and probably greater than 2.27 tons in the US [3]. The major uses of this chemical are as raw material for drugs and pesticides [1]. 3-Cyanopyridine's production and use in organic synthesis may result in its release to the environment through various waste streams [1]. There are no data available on the actual exposure levels at present. The possibility of human exposure to 3-cyanopyridine has aroused concern regarding its toxicological potential.

Only limited information is available about the toxicity of 3-cyanopyridine. It was reported that the range of oral LD50 values was 1185–1680 mg/kg in rats [4–6] and 1225 mg/kg in mice [7], and 3-cyanopyridine absorbed through intact skin caused the death of rabbits [6]. 3-Cyanopyridine showed eye irritation [6,8] and irritation of damaged skin [6], but did not irritate intact skin [9] in rabbits. Since there is insufficient information on toxicity, this chemical was selected as an object substance in an existing chemical testing program by the Japanese government [10]. In this program, an acute toxicity test, a repeated dose 28-day oral toxicity study, a bacterial reverse mutation test, and an *in vitro* mammalian chromosome aberration test were performed according to OECD test guidelines [10]. The results are briefly summarized as follows: Oral LD50s were 1475 mg/kg for male rats and 1455 mg/kg for female rats. In the 28-day repeated orally dose toxicity test with rats at doses of 0, 5, 30 and 180 mg/kg/day, the no observed effect level is considered to be 5 mg/kg/day based on increased liver and kidney weights, centrilobular hypertrophy of hepatocytes, and hyaline droplets in proximal tubules observed at 30 mg/kg/day or more. In adding to these tests in the existing chemical testing program by the Japanese government [10], a reproduction/developmental toxicity screening test was performed according to OECD test guideline 421, because the evaluation of reproductive and developmental toxicity is essential in the risk assessment of chemicals. In this paper, we report the data of the reproduction/developmental toxicity screening test of 3-cyanopyridine.

2. Materials and methods

This study was performed in compliance with OECD guideline 421 "Reproduction/Developmental Toxicity Screening Test" [11], and in accordance with the principles for Good Laboratory Practice [12,13] at the Safety Research Institute for Chemical Compounds (Sapporo, Japan). The experiment was approved by the Animal Care and Use Committee of the Safety Research Institute for Chemical Compounds, and was performed in accordance with the ethics criteria contained in the bylaws of the Committee.

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2.1. Animals and housing conditions

Crl:CD(SD) rats (8 weeks old) were purchased from Atsugi Breeding Center, Charles River Laboratories Japan, Inc. (Yokohama, Japan). This strain was chosen because it is most commonly used in toxicity studies, including reproductive and developmental toxicity studies, and historical control data are available. The animals were acclimatized to the laboratory for 13 days and subjected to treatment at 10 weeks of age. They were carefully observed during the acclimation period, and male and female rats found to be in good health were selected for use. In addition, vaginal smears of each female were recorded, and only females showing a 4- to 5-day estrous cycle were used in the experiment. Two days before the initial treatment, the rats were distributed into 4 groups of 12 males and 12 females each by stratified random sampling based on body weight.

Throughout the study, animals were maintained in an air-conditioned room set at 22 ± 3 °C, with relative humidity set at 50 ± 20%, a 12-h light/dark cycle, and ventilation with more than 10 air changes/h. A basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were provided *ad libitum*. The rats were housed individually, except for mating and nursing periods. From day 17 of pregnancy to the day of sacrifice, individual dams and/or litters were reared using wood chips as bedding (White Flake; Charles River Laboratories Japan, Inc., Yokohama, Japan).

2.2. Chemicals and doses

3-Cyanopyridine was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and kept in a cool, dark place. The 3-cyanopyridine (Lot no. GL01) used in this study was 99.9% pure, and stability during the study was verified by gas chromatography. The test article was dissolved in purified water (Otsuka Pharmaceutical Factory, Inc., Naruto, Japan), and administered to the animals by gastric intubation. Control rats received the vehicle alone. Dosing solutions were prepared at least once per 8 days and stored at room temperature until dosing, as stability under these conditions has been confirmed for up to 8 days. The concentrations of 3-cyanopyridine in the formulations were confirmed to be 98.1–101.4% of the target by HPLC analysis.

Prior to the present reproductive and developmental toxicity screening study, a repeated dose 28-day oral toxicity study was performed, as mentioned in Section 1. In this repeated dose study, male and female rats were given 3-cyanopyridine by gavage at 5, 30 or 180 mg/kg/day for 28 days. Taking into account the results of this repeated dose study, the dose levels of 3-cyanopyridine in the present study were set as 5, 30 or 180 mg/kg/day. The daily dose volume (5 ml/kg body weight) was calculated according to the latest body weight.

2.3. Study design

Male rats were dosed once daily for 42 days, beginning 14 days before mating and throughout the mating period. Female rats were also dosed once daily from 14 days prior to mating, and throughout the mating and gestation periods, to day 3 of lactation, and so the total administration period was 40–53 days. The day of the first dosing was designated as day 0 of the administration/premating period.

During the first 14-day administration period (premating period), vaginal lavage samples of each female were evaluated daily for estrous cyclicity. After this premating period, female rats were transferred to the home cage of a male in the same group, and cohabited on a 1:1 basis until successful copulation occurred or the mating period of 2 weeks had elapsed. Estrous cycles of 4–5 days were judged as normal, cycles other than 4–5 days were judged as irregular and, in particular, cycles with more than 7 days diestrus were judged as continuous diestrus. An irregular estrous cycle and a cycle with continuous diestrus were counted as abnormal. The mean estrous cycle of each treatment group was calculated without the data from females with continuous diestrus. During the mating period, vaginal smears were examined daily for the presence of sperm, and the presence of sperm in the vaginal smear and/or a vaginal plug were considered as evidence of successful mating. The day of successful mating was designated as day 0 of pregnancy. The first day when pups are found was made into the day of the initiation of delivery. Pregnant females were allowed to deliver spontaneously and nurse their pups, and the day on which the delivery was completed by 9:00 was designated as day 0 of lactation or postnatal day (PND) 0.

Throughout the study, all parental animals were observed for clinical signs of toxicity at least three times a day. Body weight was recorded on days 0, 1, 4, 6, 9, 13, 20, 27, 34 and 41 of the dosing period, and on the day of necropsy in males, and on days 0, 1, 4, 6, 9 and 13 of the premating period, on days 0, 1, 3, 5, 7, 10, 14, 17 and 20 of the gestation period and on days 0, 1 and 4 (the day of necropsy) of the lactation period in females. Body weight was additionally recorded on days 20, 27, 34, 41, 48, 50 and 51 (the day of necropsy) in copulation failure females. Food consumption was recorded on the same day as body weights in males and females, except for the mating period and necropsy day.

All surviving male rats were euthanized by exsanguination under ether anesthesia on the day after the last administration. All female rats showing successful reproductive performance were euthanized in a similar way on day 4 of lactation. Females that did not copulate were euthanized on the day after the 51st administration. Females that had not completed parturition were euthanized the day after day 25 of gestation. When total litter loss and abnormal delivery were observed, the dams were euthanized immediately. For all parental animals, the external surfaces were examined. The abdomen and thoracic cavity were opened, and gross internal examination was performed. For females, the numbers of corpora lutea and implantation sites were recorded. The following organs were weighed: liver, kidneys, spleen and adrenal gland in both sexes, the testes and epididymides in males, and ovaries in females.

Histopathological evaluations were performed on the liver in both sexes, on the testes, epididymides, prostate gland, and seminal vesicles with coagulating gland in males, and on the ovaries in females in the control and highest dose groups. In addition, organs with macroscopically abnormal findings were examined histopathologically. Test substance-related histopathological changes were found in the liver, testes and epididymides of the highest dose group in males, and the liver weights in middle and high dose groups in males and in low and middle dose groups in females were higher than in control groups; therefore, those organs of all animals in the low and middle groups were also examined histopathologically. The stages of spermatogenesis in the testes were examined in all male groups. For the histopathological examination, the target organs, except for the testes and epididymides, were fixed in 10% neutral-buffered formalin and the testes and epididymides were fixed with Bouin's solution. Those organs were processed routinely for embedding in paraffin, and sections were prepared for staining with hematoxylin–eosin.

All live and dead pups were counted, sexed and examined grossly on PND 0. Live pups were weighed per sex per litter on PNDs 0, 1 and 4. They were observed daily for clinical signs of toxicity on PNDs 0–4. On PND 4, the pups were euthanized by CO_2 inhalation, and gross internal examinations were performed.

2.4. Data analysis

Parametric data, such as body weight, body weight gain, food consumption, organ weight, the number of germ cells in each spermatogenic stage in the testes, estrous cycle length, the number of corpora lutea, the number of implantations, implantation index, the numbers of pups born, live pups and dead pups on lactation day 0, delivery index, live birth index, sex ratio, gestation length, and the number of live pups and viability index on lactation day 4 were analyzed by Bartlett's test for homogeneity of distribution. When homogeneity was recognized, one-way analysis of variance was performed. If a significant difference was detected, Dunnett's test was conducted for comparisons between control and individual treatment groups. Data without homogeneity or histopathological findings with two grades or more were analyzed using the Kruskal–Wallis rank sum test. If significant differences were found, the Mann–Whitney *U*-test was conducted for comparison between the control and each dosage group. The incidence of abnormal estrous cycle, copulation index, fertility index, live birth index, nursing index on lactation day 4, and histopathological findings with one grade were analyzed using the multi-sample chi-square test. If significant differences were found, the chi-square test or Fisher's exact test was conducted for comparison between the control and each dosage group. Pup data were statistically analyzed using the litter as the experimental unit. The 5% level of probability was used as the criterion for significance.

3. Results

3.1. Parental toxicity

No substance-related clinical signs of toxicity were detected at 5 and 30 mg/kg/day in either sex. Soft feces in two males, swelling of the left hindlimb in one male and salivation in one male were observed at 180 mg/kg/day. Alopecia in one female in the premating and mating periods, and two females in the gestation period, salivation in one female and pale skin in one female in the gestation period were observed at 180 mg/kg/day. At 180 mg/kg/day, moreover, two pregnant females were debilitated, and were found dead on gestation day 21 (prior to the initiation of delivery) and 24 (during delivery), respectively, and two delivering females were euthanized moribund on gestation day 25. One delivered female was euthanized at the end of delivery on gestation day 25 because of complete stillbirth. Three pregnant females, which did not deliver until gestation day 25, were euthanized on gestation day 26. Two delivered females were euthanized because their pups were all dead on PNDs 0 or 1. No substance-related clinical signs of toxicity were detected at 180 mg/kg/day in two females with copulation failures.

Body weight and the gain in each group are shown in Table 1. At 180 mg/kg/day, body weight was significantly reduced on days 4–13 of the dosing period in males. In females, body weight was significantly reduced on days 4–9 of the premating period and on days 5–20 of the gestation period, and body weight gain during the gestation period was significantly decreased at 180 mg/kg/day. The increased body weight values at 30 mg/kg/day were not considered toxicological effects in the present study. Food consumption was significantly decreased on days 1–6 of the administration period at 180 mg/kg/day in males, and on days 4–9 of the premating period and days 5–10 of the gestation period at 180 mg/kg/day in females (data not shown).

At necropsy (data not shown in tables), in males, enlargement of the liver was observed in two animals at 30 mg/kg/day and in 12 animals at 180 mg/kg/day, and a yellowish-white patch/mass in the epididymis was observed in each three animals at 30 and 180 mg/kg/day. Moreover, diverticulum in the ileum in one animal, atrophy in the right testis and epididymis in one animal, and swelling of the left hindlimb in one animal were observed at 180 mg/kg/day. In females, diverticulum in the ileum was observed in one animal at 30 mg/kg/day, and thickening of the wall in the ileum was observed in one animal at 180 mg/kg/day. In one female at 180 mg/kg/day, which died on gestation day 24, a black patch in the mucosa of the stomach and atrophy of the spleen were observed.

Absolute and relative organ weights of scheduled-sacrifice animals in each group are shown in Table 2. Absolute and relative weights of the liver were significantly increased in males at 30 mg/kg/day or more. Absolute and relative weights of the kidney and adrenal gland were significantly increased, and absolute weight of the epididymis was significantly decreased in males at 180 mg/kg/day. Absolute testis weight was decreased 8% from control at 180 mg/kg/day, but insignificantly. In females at 180 mg/kg/day, the organ weights were not statistically analyzed because ten died or were euthanized in the gestation or lactation periods. Relative weights of the liver were significantly increased in females at 5 and 30 mg/kg/day, and absolute weight of the liver was significantly increased in females at 30 mg/kg/day.

Histopathological findings are shown in Table 3. The incidence of centrilobular hepatocyte hypertrophy in the liver was significantly increased in males at 30 mg/kg/day or more. Incidences of Sertoli cell vacuolation, spermatid necrosis and spermatid decrease in the testis, and incidences of spermatozoa decrease and lumen cell debris in the epididymis were significantly increased in males at 180 mg/kg/day. In females, in the liver, centrilobular hepatocyte hypertrophy, extramedullary hematopoiesis, periportal fatty change and focal necrosis were observed at 180 mg/kg/day. Values at 180 mg/kg/day in females were excluded from statistical evaluation because no animal survived at terminal euthanasia. The incidence of centrilobular hepatocyte hypertrophy in the liver was significantly increased in females at 30 mg/kg/day.

3.2. Stages of spermatogenesis in the testis

The number of Sertoli cells and the number of germ cells per Sertoli cell in each stage of spermatogenesis in the testes are shown in Table 4. The number of pachytene spermatocytes and the number of round spermatids were significantly decreased at 180 mg/kg/day in stages I–VI. In stages VII–VIII, the number of preleptotene spermatocytes was significantly decreased at 30 mg/kg/day or more, and the number of pachytene spermatocytes and the number of round spermatids were significantly decreased at 180 mg/kg/day. The number of spermatogonia, the number of zygotene/pachytene spermatocytes and the number of pachytene/diplotene spermatocytes were significantly decreased at 180 mg/kg/day in stages XII–XIV.

3.3. Reproductive findings

The reproductive findings are shown in Tables 5 and 6. Reproduction performance of parental rats, delivery and nursing were not significantly different between the control and 5 or 30 mg/kg/day groups. Continuous diestrus was observed in one female each at 5 and 30 mg/kg/day and in two females at 180 mg/kg/day, and an irregular estrous cycle was observed in one female at 30 mg/kg/day and in seven females at 180 mg/kg/day. At 180 mg/kg/day, the mean estrous cycle was significantly prolonged, the incidence of abnormal estrous cycle was 66.7%, the initiation of delivery was significantly delayed, and the gestation index was significantly decreased. Two pairs at 180 mg/kg/day did not copulate. Although additional mating was carried out with males that already succeeded in copulating with other females, copulation with these two

Table 1
Body weight of male and female rats given 3-cyanopyridine by gavage.

Dose (mg/kg/day)	No. of males	Day 0	Day 1	Day 4	Day 6	Day 9	Day 13	Day 20	Day 27	Day 34	Day 41	Gain
Body weight during administration (g)												
0	12	375.8 ± 18.4	378.3 ± 17.4	391.9 ± 20.4	399.0 ± 22.3	409.2 ± 24.4	421.5 ± 26.4	438.6 ± 23.6	458.6 ± 27.4	476.8 ± 29.8	491.2 ± 32.2	115.3 ± 19.4
5	12	375.9 ± 15.4	379.9 ± 16.4	393.7 ± 19.4	401.6 ± 20.7	413.7 ± 22.0	429.2 ± 24.8	450.9 ± 25.7	471.4 ± 29.8	494.3 ± 32.2	511.2 ± 33.8	135.3 ± 22.2
30	12	375.2 ± 15.2	377.6 ± 14.1	394.3 ± 15.5	402.9 ± 16.7	417.5 ± 17.3	433.3 ± 19.8	456.8 ± 20.4	481.2 ± 23.8	504.8 ± 27.6	524.7 ± 26.8*	149.5 ± 19.2**
180	12	374.3 ± 13.8	369.2 ± 11.0	328.3 ± 14.4**	333.6 ± 18.9**	362.9 ± 14.3**	385.4 ± 11.7**	414.5 ± 29.5	438.8 ± 26.1	462.8 ± 33.3	477.7 ± 37.3	103.4 ± 36.2

Dose (mg/kg/day)	No. of females	Day 0	Day 1	Day 4	Day 6	Day 9	Day 13	Gain
Body weight during premating (g)								
0	12	256.3 ± 12.4	254.0 ± 11.9	263.6 ± 12.6	266.5 ± 16.1	273.2 ± 16.7	282.3 ± 15.8	25.9 ± 10.0
5	12	252.8 ± 12.5	255.3 ± 12.7	261.9 ± 14.3	267.3 ± 13.4	273.8 ± 15.9	280.1 ± 15.9	27.3 ± 11.3
30	12	250.5 ± 10.8	254.8 ± 11.7	260.1 ± 13.4	268.0 ± 14.4	274.6 ± 15.8	279.6 ± 16.1	29.1 ± 8.1
180	12	248.2 ± 16.0	248.0 ± 14.7	234.1 ± 18.6**	233.7 ± 25.5**	247.6 ± 21.6**	271.3 ± 15.7	23.1 ± 14.2

Dose (mg/kg/day)	No. of females	Day 0	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 17	Day 20	Gain
Body weight during gestation (g)											
0	12	289.7 ± 18.2	296.2 ± 17.4	311.3 ± 17.9	320.9 ± 18.0	331.8 ± 20.0	348.9 ± 19.4	371.6 ± 21.3	402.7 ± 21.2	448.3 ± 24.4	158.6 ± 21.8
5	12	291.5 ± 18.6	297.6 ± 16.7	314.8 ± 18.7	324.5 ± 19.6	333.1 ± 20.6	348.9 ± 21.2	372.7 ± 23.9	402.8 ± 26.1	454.3 ± 30.4	162.8 ± 23.0
30	12	289.3 ± 18.9	295.9 ± 19.3	311.3 ± 19.7	321.3 ± 19.9	329.1 ± 19.7	345.7 ± 22.2	368.2 ± 24.0	396.7 ± 24.2	447.9 ± 29.1	158.6 ± 17.0
180	10	275.1 ± 18.1	280.3 ± 21.0	293.9 ± 21.8	300.2 ± 21.5*	306.2 ± 20.6*	316.2 ± 19.3**	337.0 ± 25.2**	357.0 ± 23.8**	371.4 ± 34.1**	96.3 ± 29.3**

Dose (mg/kg/day)	No. of females	Day 0	Day 1	Day 4	Gain
Body weight during lactation (g)					
0	12	347.8 ± 26.3	354.1 ± 25.8	366.8 ± 21.6	19.1 ± 12.5
5	12	343.8 ± 29.6	347.8 ± 30.0	366.7 ± 28.2	22.8 ± 6.1
30	12	335.8 ± 27.9	345.0 ± 28.9	365.2 ± 22.7	29.4 ± 7.6*
180	2	297.5 ± 7.8	–	–	–

Values are given as the mean ± S.D. (–) Blank.
Body weights on day 0 of the lactation period in the 180 mg/kg/day group are excluded from statistical evaluation because of insufficient sample numbers.
* Significantly different from the control group ($P < 0.05$).
** Significantly different from the control group ($P < 0.01$).

Table 2
Organ weight of male and female rats given 3-cyanopyridine by gavage.

Dose (mg/kg/day)	No. of males	Body weight	Liver (g)	Kidney (g)	Spleen (g)	Adrenal (mg)	Testis (g)	Epididymis (g)
0	12	493.9 ± 32.8	15.987 ± 1.453	3.297 ± 0.240	0.812 ± 0.115	57.8 ± 7.7	3.338 ± 0.183	1.353 ± 0.087
			(3.240 ± 0.248)	(0.670 ± 0.049)	(0.164 ± 0.018)	(11.765 ± 1.800)	(0.678 ± 0.062)	(0.275 ± 0.024)
5	12	514.8 ± 32.4	16.822 ± 1.354	3.206 ± 0.240	0.840 ± 0.154	57.3 ± 8.5	3.338 ± 0.243	1.388 ± 0.089
			(3.272 ± 0.223)	(0.625 ± 0.043)	(0.163 ± 0.026)	(11.162 ± 1.750)	(0.650 ± 0.060)	(0.269 ± 0.025)
30	12	527.7 ± 26.7*	19.979 ± 1.941**	3.563 ± 0.433	0.843 ± 0.148	57.9 ± 9.7	3.240 ± 0.253	1.323 ± 0.132
			(3.781 ± 0.216**)	(0.673 ± 0.059)	(0.159 ± 0.024)	(10.951 ± 1.577)	(0.615 ± 0.041)	(0.250 ± 0.020)
180	12	481.3 ± 35.8	24.007 ± 1.599**	3.940 ± 0.302**	0.743 ± 0.097	74.5 ± 13.1**	3.068 ± 0.387	1.228 ± 0.205*
			(5.002 ± 0.327**)	(0.821 ± 0.045**)	(0.153 ± 0.021)	(15.583 ± 3.145**)	(0.642 ± 0.088)	(0.256 ± 0.037)

Dose (mg/kg/day)	No. of females	Body weight	Liver (g)	Kidney (g)	Spleen (g)	Adrenal (mg)	Ovary (mg)
0	12	366.8 ± 21.6	13.374 ± 1.001	2.118 ± 0.243	0.787 ± 0.195	81.3 ± 10.3	118.0 ± 9.3
			(3.650 ± 0.243)	(0.578 ± 0.058)	(0.215 ± 0.049)	(22.202 ± 3.007)	(32.183 ± 1.951)
5	12	366.7 ± 28.2	14.195 ± 1.369	2.223 ± 0.134	0.854 ± 0.213	78.8 ± 6.4	121.0 ± 8.7
			(3.872 ± 0.200*)	(0.608 ± 0.043)	(0.234 ± 0.061)	(21.578 ± 2.276)	(33.117 ± 2.853)
30	12	365.2 ± 22.7	15.220 ± 0.953**	2.203 ± 0.158	0.857 ± 0.088	77.3 ± 8.0	121.1 ± 15.0
			(4.178 ± 0.253**)	(0.603 ± 0.047)	(0.235 ± 0.026)	(21.177 ± 2.095)	(33.185 ± 3.857)
180	0	–	–	–	–	–	–

Values are given as the mean ± S.D. Values in parentheses are relative organ weights (g or mg/100 g body weight).
* Significantly different from the control group ($P < 0.05$).
** Significantly different from the control group ($P < 0.01$).

females was not observed. These two unsuccessfully mated females (the length of the estrous cycle of one was 5.0 days and that of the other was not calculated due to continuous diestrus) were alive and were euthanized on the next day of administration, day 51. The other ten females at 180 mg/kg/day, which had succeeded in copulation, became pregnant, and the fertility index was not significantly different between the control and 180 mg/kg/day groups. At 180 mg/kg/day, one pregnant female was found dead on gestation day 21 prior to the initiation of delivery, one pregnant female became exhausted in the middle of delivery on gestation day 23, equal to the day of the initiation of delivery, and was found dead the next day, and two pregnant females became exhausted in the middle of delivery on gestation day 25, equal to the day of the initiation of delivery, and were euthanized because of difficult delivery. For these four pregnant females, dead fetuses were observed in their uterus at necropsy. One pregnant female was successfully delivered on gestation day 25 but all her pups were stillborn. Three pregnant females had not delivered by gestation day 25, were euthanized on gestation day 26, and dead fetuses were observed in their uterus at necropsy. There were no abnormal findings in the nursing period for two pregnant females successfully delivered on gestation day 23 or 24, and these females were euthanized because their newborn pups all died on lactation day 0 or 1. Because of insufficient sample numbers due to such abnormal delivery or pup death, the gestation length, delivery index, nursing index, and litter data on the lactation days 0 and 4 were excluded from statistical evaluation in the 180 mg/kg/day group.

3.4. Developmental findings

The developmental findings are shown in Tables 6 and 7. At 180 mg/kg/day, the numbers of corpora lutea and implantations were significantly decreased. The numbers of dead or missing pups between PNDs 0 and 4 were one male and one female in controls, four males and one female at 5 mg/kg/day, two males and four females at 30 mg/kg/day, and 19 males and 11 females at 180 mg/kg/day, including pups born by abnormal delivery. For live pups on PND 4, trauma or a scab on the tip of the tail/loss of tail was observed in one female in controls. Regarding general appearances, there were no abnormal findings at 5 and 30 mg/kg/day. At 180 mg/kg/day, general edema was observed in all pups, four males and three females, alive on PND 0. There were no significant differences in the sex ratio of live pups, the viability index on PND 4, and the body weight of male and female pups on PNDs 0 and 4 between the control group and the treated group at 5 or 30 mg/kg/day.

At necropsy of dead pups between PNDs 0 and 4, there were no abnormal findings at 0, 5 and 30 mg/kg/day. At 180 mg/kg/day, general edema in 16 males and 11 females, ascites of the abdominal cavity in seven males and four females, hydrothorax of the thoracic cavity in two males, omphalocele in one female, and pale discoloration of the lung and heart and grayish-green discoloration and deformity of the liver in one male were observed. At necropsy of live pups on PND 4, yellowish-brown discoloration of the liver in one female and a grayish-green patch on the lateral left lobe of the liver and loss of the tail in one female in controls, small kidneys with dark red discoloration in one male, and dilatation of the renal pelvis in the kidneys in one female at 5 mg/kg/day were observed. These findings of live pups on PND 4 at 5 mg/kg/day were toxicologically insignificant because there were no abnormal findings in the 30 mg/kg/day group at necropsy of live pups on PND 4.

4. Discussion

The current study was conducted to examine the possible effects of 3-cyanopyridine on reproduction and development in rats. The

Table 3
Histopathological findings of male and female rats given 3-cyanopyridine by gavage.

	Grade	Dose (mg/kg/day) 0	5	30	180
Number of males examined		12	12	12	12
Liver: Hypertrophy, hepatocytes, centrilobular	+	0	0	7 *	0]**
	++	0	0	0	12]**
Fatty change, centrilobular	+	0	0	0	2
Microgranuloma	+	2	2	1	1
Testis: Vacuolation, Sertoli cell	+	0	0	0	10]**
	++	0	0	0	1]**
Necrosis, spermatid	+	0	0	1	11**
Decrease, spermatid	+	0	0	0	9**
Appearance, multinucleated giant cells	+	0	0	0	3
Atrophy, seminiferous tubule	+	0	0	4	0
	+++	0	0	0	1
Edema, interstitium	+	0	0	0	1
Epididymis: Decrease, spermatozoa	+	0	0	1	7
	++	0	0	0	1]**
	+++	0	0	0	1]**
Cell debris, lumen	+	0	0	4	9]**
	++	0	0	0	2]**
Spermatic granuloma	+	0	1	3	2
	++	0	0	0	1
Cellular infiltration, inflammatory cells, interstitium	+	0	0	0	1
Edema, interstitium	+	0	0	0	1
Atrophy, epithelium, ductus epididymis	+	0	0	0	1
Prostate: Cellular infiltration, inflammatory cells	+	4	-	-	1
	++	2	-	-	0
Ileum: Diverticulum	+	-	-	-	1 (1)
Hind limb: Callus formation	++	-	-	-	1 (1)
Proliferation, osteoclasts, bone marrow	+	-	-	-	1 (1)
Granulation, articular capsule	+	-	-	-	1 (1)
Cellular infiltration, inflammatory cells, subcutis	++	-	-	-	1 (1)
Edema, subcutis	+++	-	-	-	1 (1)

	Grade	Dose (mg/kg/day) 0	5	30	180
Number of females examined		12	12	12	12
Liver: Hypertrophy, hepatocytes, centrilobular	+	0	0	5 *	5
	++	0	0	0	6
Extramedullary hematopoiesis	+	0	0	0	2
Fatty change, periportal	+	0	0	0	2
Necrosis, focal	+	0	1	0	1
Microgranuloma	+	0	0	1	0
Forestomach: Erosion	+	-	-	-	1 (1)
Cellular infiltration, inflammatory cell, submucosa	+	-	-	-	1 (1)
Edema, submucosa	++	-	-	-	1 (1)
Glandular stomach: Erosion	+	-	-	-	1 (1)
Ileum: Diverticulum	+	-	-	1 (1)	0 (1)
Cellular infiltration, inflammatory cells, lamina propria and submucosa	++	-	-	0 (1)	1 (1)
Spleen: Atrophy white pulp	++	-	-	-	1 (1)
Skin: Atrophy, hair follicle	+	1 (1)	-	-	1 (1)

Values are the number of animals with findings. Values in parentheses are the number of animals examined. -: Blank.
Grade: +, slight change; ++, moderate change; +++, severe change.
*Significantly different from the control group ($P < 0.05$). **Significantly different from the control group ($P < 0.01$).
Values of females in the 180 mg/kg/day group are excluded from statistical evaluation.

Values are the number of animals with findings. Values in parentheses are the number of animals examined. (–) Blank. Grade: (+) slight change; (++) moderate change; (+++) severe change. Values of females in the 180 mg/kg/day group are excluded from statistical evaluation.
*Significantly different from the control group ($P < 0.05$).
**Significantly different from the control group ($P < 0.01$).

Table 4
Stages of spermatogenesis of male rats given 3-cyanopyridine by gavage.

Dose (mg/kg/day)	Number of animals	Number of SC	Number of spermatogonia/SC	Number of preleptotene spermatocytes/SC	Number of pachytene spermatocytes/SC	Number of round spermatids/SC
Stage I–VI						
0	12	22.50 ± 1.55	0.874 ± 0.139		2.393 ± 0.248	6.151 ± 0.493
5	12	22.15 ± 1.69	0.874 ± 0.200		2.299 ± 0.183	6.309 ± 0.352
30	12	22.25 ± 1.80	0.838 ± 0.214		2.214 ± 0.191	5.958 ± 0.614
180	12	21.47 ± 1.83	0.773 ± 0.253		2.113 ± 0.176**	5.173 ± 0.840**
Stage VII–VIII						
0	12	21.27 ± 1.33	0.092 ± 0.016	2.041 ± 0.209	2.818 ± 0.317	6.143 ± 0.454
5	12	21.30 ± 1.61	0.083 ± 0.021	2.007 ± 0.222	2.794 ± 0.256	6.344 ± 0.408
30	12	21.17 ± 1.43	0.091 ± 0.028	1.819 ± 0.210*	2.666 ± 0.206	5.880 ± 0.549
180	12	21.37 ± 1.87	0.067 ± 0.037	1.668 ± 0.213**	2.330 ± 0.265**	4.693 ± 0.742**

Dose (mg/kg/day)	Number of animals	Number of SC	Number of spermatogonia/SC	Number of leptotene spermatocytes/SC	Number of pachytene spermatocytes/SC
Stage IX–XI					
0	12	23.47 ± 1.80	0.172 ± 0.050	2.173 ± 0.235	2.793 ± 0.254
5	12	22.18 ± 1.49	0.207 ± 0.073	2.342 ± 0.239	2.777 ± 0.234
30	12	22.77 ± 2.47	0.166 ± 0.032	1.987 ± 0.202	2.613 ± 0.252
180	12	21.55 ± 1.88	0.140 ± 0.036	1.994 ± 0.251	2.587 ± 0.310

Dose (mg/kg/day)	Number of animals	Number of SC	Number of spermatogonia/SC	Number of spermatocytes 1/SC	Number of spermatocytes 2/SC
Stage XII–XIV					
0	12	22.97 ± 1.20	0.202 ± 0.046	2.432 ± 0.151	3.006 ± 0.197
5	12	23.05 ± 1.13	0.188 ± 0.052	2.314 ± 0.138	3.015 ± 0.172
30	12	21.83 ± 1.87	0.180 ± 0.042	2.309 ± 0.181	2.940 ± 0.162
180	12	22.72 ± 2.04	0.141 ± 0.047**	2.032 ± 0.213**	2.641 ± 0.332**

SC: Sertoli cell. Spermatocytes 1: zygotene or pachytene spermatocytes. Spermatocytes 2: pachytene or diplotene spermatocytes. Values are given as the mean ± S.D.
* Significantly different from the 0 mg/kg group at $P < 0.05$.
** Significantly different from the 0 mg/kg group at $P < 0.01$.

dosage of 3-cyanopyridine used in this study was sufficiently high to be expected to induce general toxic effects in parental animals. As expected, many general toxic effects were observed. The following results suggest that the liver is a sensitive target organ. The weights of the liver were increased in males at 30 mg/kg/day or more and in females at 5 and 30 mg/kg/day, and the incidence of centrilobular hepatocyte hypertrophy in the liver was increased in both sexes at 30 mg/kg/day or more. These findings in the liver were

Table 5
Reproductive findings in rats given 3-cyanopyridine by gavage.

	Dose (mg/kg/day)			
	0	5	30	180
No. of pairs	12	12	12	12
Estrous cycles (day)[a]	4.03 ± 0.09	4.00 ± 0.00	4.09 ± 0.22	5.17 ± 0.48**
Abnormal estrous cycle[b]	0.0	8.3	16.7	66.7**
No. of pairs with successful copulation	12	12	12	10
Copulation index (male/female)[c]	100/100	100/100	100/100	83.3/83.3
Fertility index[d]	100	100	100	100
Gestation index[e]	100	100	100	22.2 (9)[g]**
Initiation of delivery (date)[a]	21.7 ± 0.5	21.8 ± 0.5	21.5 ± 0.5	24.0 ± 0.9 (6)[h]**
No. of females completing the delivery	12	12	12	3
Gestation length (day)[a]	22.3 ± 0.5	22.1 ± 0.3	22.4 ± 0.5	[23.5 ± 0.7]
No. of dams delivering live pups	12	12	12	2
Nursing index[f]	100	100	100	[0]

Values in brackets are excluded from statistical evaluation because of insufficient sample numbers.
[a] Values are given as the mean ± S.D.
[b] Abnormal estrous cycle (%) = no. of females with abnormal estrous cycle/no. of females with normal estrous cycle × 100.
[c] Copulation index (%) = no. of copulated rats/no. of pairs × 100.
[d] Fertility index (%) = no. of pregnant females/no. of pairs with successful copulation × 100.
[e] Gestation index (%) = no. of dams with live pups/no. of pregnant females × 100.
[f] Nursing index = no. of females nursing live pups on lactation day 4/no. of females with live pups delivery × 100.
[g] No. of animals was 9 because one pregnant female was found dead on gestation day 21 before delivery.
[h] No. of animals was 6 because one was found dead on gestation day 21 before delivery and three had not delivered by gestation day 25 in 10 pregnant females.
** Significantly different from the control group ($P < 0.01$).