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Research Article

Quinoline (Cas: 91-22-5) Effect on Pubertal Development and Thyroid Function in the Intact Peripubertal Female Rats

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ABSTRACT
Quinoline potential effects on pubertal development and thyroid function were quantified in the peripubertal female Wistar rats. A total of 60 peripubertal rats were divided into 4 groups comprised of 15 rats/group. Vehicle control (corn oil – 0 mg/kg), positive control – 1-chloro-2-nitrobenzene (100 mg CNB/kg) and two groups of Quinoline (QNL – 100 and 150/200) mg/kg were administered, from postnatal day (PND) 22–42 at the dose volume of 2.5 mL/kg. All animals were sacrificed approximately 2 hours following the last dose on PND 42. No treatment related mortality was observed in control, CNB, and 100 mg/kg QNL. At 200 mg/kg QNL, two mortalities, and a significant reduction in body weight were observed hence dose was reduced to 150 mg/kg from PND 25. Weakness and lethargy were observed in 150/200 mg/kg QNL group. Body weight, body weight gain, and feed consumption of the 100 and 150 mg/kg QNL groups were significantly decreased, compared to the vehicle control group. Body weight, body weight gain, and feed consumption of the positive control group were comparable with the vehicle control group. Out of 15 rats/group – 13 (G1), 5 (G2), 11 (G3), and 4 (G4) rats had complete vaginal opening. Terminal body weight of the 150 mg/kg QNL groups was significantly decreased, as compared to the vehicle control group. Absolute liver weight of CNB and QNL treated groups were significantly increased compared to the vehicle control group. Absolute organ weights (pituitary, adrenals, thyroid and parathyroid) of QNL treated groups were comparable to the vehicle control group. Thyroid hormone levels (T4 and TSH) of QNL treated groups were comparable. Based on the result of study, QNL had not altered pubertal development and thyroid function in peripubertal rats.

Keywords: Quinoline, Maximum Tolerated Dose, Thyroid Hormone

INTRODUCTION

In the 1990's, scientists put forward that certain chemicals might be disrupting the endocrine system of human and animals. A variety of chemicals have been found to disrupt the endocrine system of animals in laboratory studies, and compelling evidence shows that endocrine system of certain fish and wildlife has been affected by chemical contaminants, resulting in developmental and reproductive problems. With reference to the US EPA 1996[1] and based on above mentioned findings, section 408(p) of the 1996 Food Quality Protection Act (FQPA) mandated the US Environmental Protection Agency (EPA) to develop and maintain a screening program to investigate the potency of chemicals to interfere with the endocrine system in human. After passage of the FQPA, EPA convened a meeting with the Federal Advisory Committee, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), to assess the current state of the science and assist in developing an endocrine screening program[2]. Based largely on the EDSTAC[3] recommendation, EPA developed a two-tiered framework for the Endocrine Disruptor Screening Program (EDSP): Tier 1 and Tier 2. In EDSP, Endocrine disruptors (ED) are defined as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function[4,5]. In EDSP, Tier 1 screening data is used to identify substances that have the potential to interact with the endocrine system. Chemicals, which go through Tier 1 screening and are found to exhibit the potential to interact with the estrogen, androgen, or thyroid hormone systems, will proceed to Tier 2 for testing. Tier 2 testing data identifies any adverse endocrine related effects caused by the substance, and establish a quantitative relationship between the doses and observed endocrine related adverse effect. The results of Tier 2 testing will be combined with other hazardous information and exposure assessment on a given chemical resulting in the risk assessment. Tier 1 is composed of a screening battery of 6 *in vivo* {Uterotrophic (rat); Hershberger (rat); Pubertal female (rat); Pubertal male (rat); Amphibian metamorphosis (frog); Fish short-term reproduction} and 5 *in vitro* {Estrogen receptor (ER) binding – rat uterine cytosol; Estrogen receptor - (hERα) transcriptional activation - Human cell line (HeLa-9903); Androgen

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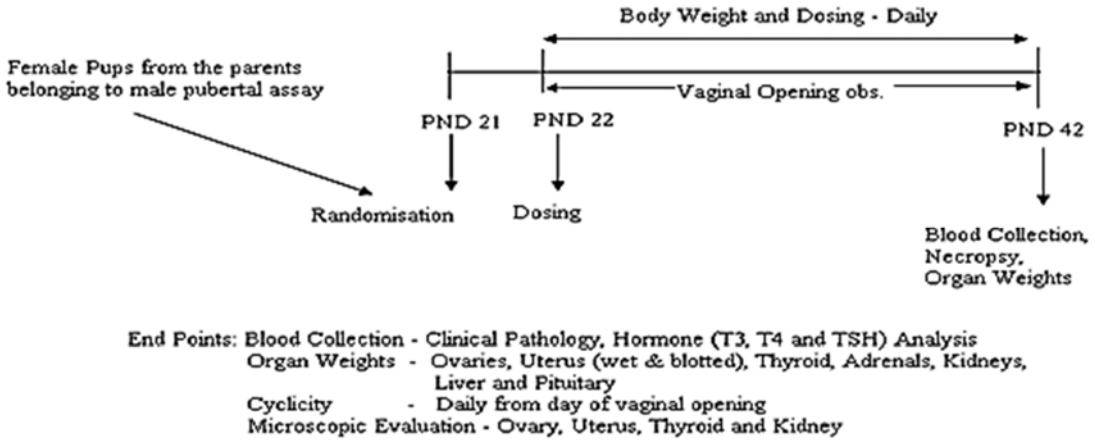

Figure 1: Female Pubertal Assay - Study Design

Table 1: Body weight on PND 21 and Weekly body weight (g) during treatment period

PND	G1 – 0 mg/kg		G2 – 100 mg CNB/kg		G3 -100 mg QNL/kg		G4 – 150/200 mg/QNL/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
21	46.49	1.79	46.63	1.79	46.53	1.7	46.39	1.52
22-28	59.44	8.77	55.87**	55.87	52.92**	5.70	47.29**	1.49
29-35	92.54	10.65	88.46	11.31	83.06**	10.94	64.39**	8.93
36-42	122.97	7.48	122.69	9.04	117.22	9.29	95.38**	95.38

Note: ** = Means different from control at p<0.01

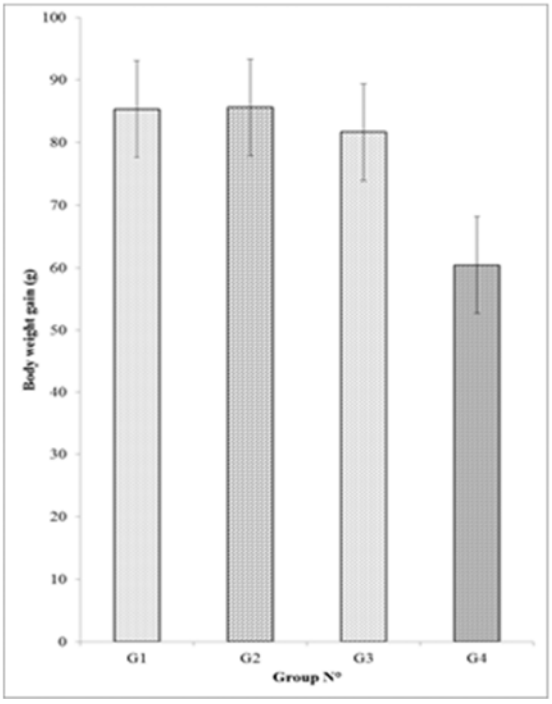

Figure 2: Body weight gain (g) from PND 22-42

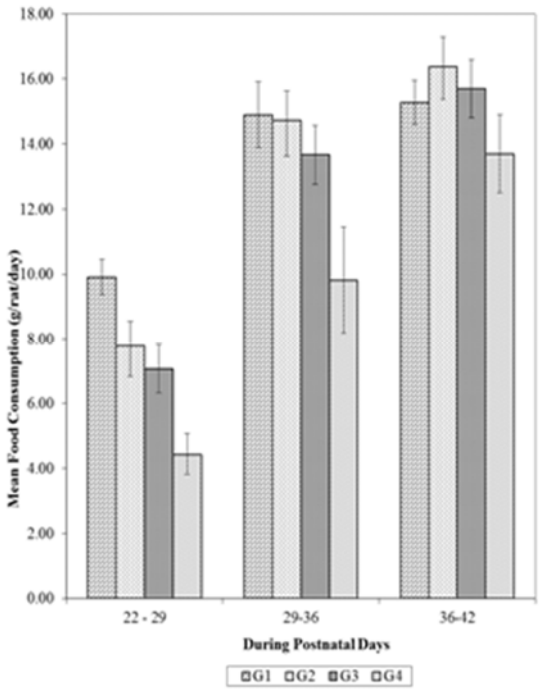

Figure 3: Feed consumption (g/rat/day)

receptor (AR) binding - rat prostate cytosol; Steroidogenesis - Human cell line (H295R); Aromatase - Human recombinant microsomes} screening assays. The Uterotrophic and Hershberger assays are used mainly to demonstrate the agonistic and antagonistic nature of chemicals. Pubertal assays provide the information about the potency of chemicals for multiple endocrine mechanism and their effects on different endocrine endpoints. Therefore, these assays can be used to provide NO(A)ELs/LO(A)ELs, to be used in human risk

Table 2: Vaginal Opening Data

Group N° with dose		G1 – 0 mg/kg		G2 – 100 mg CNB/kg		G3 -100 mg QNL/kg		G4 – 150/200 mg/QNL/kg	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age at Incomplete VO (PND)	U	34.20	1.97	35.57	1.79	35.71	2.76	38.38**	2.56
	A	34.20	2.28	35.59	2.29	35.71	2.28	38.33**	2.29
Age at VO (PND)	U	39.87	2.80	41.40	2.47	40.80	2.57	42.13	1.81
	A	39.86	1.26	41.42	1.26	40.80	1.26	42.11	1.35
Body weight at VO (g)	U	125.20	15.07	120.52	6.08	122.06	9.20	93.20**	7.84
	A	126.03	10.90	120.08	10.90	120.87	10.92	93.50**	10.84

Note: ** = Means different from control at $p<0.01$

assessment and for classification. The male and female pubertal assays are designed to detect endocrine active compounds that operate through a variety of modes of action (MoAs), including potential estrogenic/antiestrogenic effects (primarily the female assay), androgen/antiandrogen effects (primarily the male assay), modulation of steroid biosynthesis, alterations in the hypothalamic–pituitary–gonadal axis, and thyroid perturbations[6]. EPA identified the first group of 67 chemicals for testing which included pesticide active ingredients and High Production Volume (HPV) chemicals used as pesticide inert ingredients. In November 2010, the EPA published a second list of chemicals for the Endocrine Disruptor Screening Program (EDSP)[7]. New to this list were those pesticides identified by the FDA and priority pollutants under the Safe Drinking Water Act that did not appear on the first list. The second list included a large number of pesticides, two perfluorocarbon compounds and three pharmaceuticals. Quinoline (QNL) is a heterocyclic aromatic organic compound. It is a colorless hygroscopic liquid with a strong odor. QNL was first extracted from coal tar in 1834 by Friedlieb Ferdinand Runge[8,9]. Coal tar remains the principal source of commercial QNL[10]. Like other nitrogen heterocyclic compounds, such as pyridine derivatives, QNL is often reported as an environmental contaminant associated with facilities processing oil shale or coal, and has also been found at legacy wood treatment sites[11]. In the present study, to investigate changes in reproductive functions caused by QNL, experiments were performed to determine changes in body, ovary, and uterus weights, and the time of vaginal patency, after its exposure in female rat.

MATERIALS AND METHODS

Quinoline (CAS number 91-22-5), 1-chloro-2-nitrobenzene (CAS number 88-73-3) and corn oil were purchased from Sigma-Aldrich, USA. Purity of quinoline, and 1-chloro-2-nitrobenzen were 99.9% and 99.0% respectively. Purity was provided by supplier.

Animals

Rat (*Rattus* norvegicus) of Wistar strain for this project were approved by Institutional Animals Ethics Committee (IAEC) of Jai Research Foundation, Valvada, Gujarat which is AAALAC accredited facility, India. Male and female rats were taken from Animal breeding facility, Jai Research Foundation, Valvada, India. This group of animals was identified as parental animals. After acclimatization period of 6 days, male and female animals were mated in 1: 1 ratio to obtain F_1 offspring. Only those animals which were delivered by gestation day 22 or 23 and had litter size of more than eight pups/litter were considered for selection of pups for randomization. Animals which were having litter size of more than eight pups were standardized to eight pups/litter. On lactation day 21, pups body weight was recorded. The female pups were assigned to treatment groups such that the mean body weights and variances for all groups were similar.

Dose Range Finding Study

14-day repeated dose toxicity was performed to determine the dose levels for endocrine *in vivo* studies. 5 animals/sex/group (groups: 1 control group and 3 QNL treated groups; low dose – 50, mid dose – 100, and high dose – 200 mg/kg body weight/day) were dosed daily for consecutive 14 days. Body weight and feed consumption were recorded twice weekly. Blood was withdrawn through retro orbital plexus for estimation of clinical biochemistry parameters. Testes, epididymes, LABC, glance penis, ventral prostate, dorsolateral prostate, ovary, and liver were weighed.

Experimental Design

In this assay, pups of postnatal day 21, were divided into four groups. Each group had 15 pups of approximately equal body weight and covariance. Furthermore, pups were blocked by litter to ensure that littermates were not assigned to the same treatment groups[12].

Group Design

Group-1: Vehicle control (Corn oil; 0 mg/kg body weight/day)

Group-2: Positive control (1-chloro-2-nitrobenzene; CNB; 100 mg/kg body weight/day)

Group-3: Low dose (Quinoline; QNL; 100 mg/kg body weight/day)

Group-4: High dose (Quinoline; QNL; 150/200 mg/kg body weight/day)

Observation

All animals were examined twice daily for toxic clinical sign throughout treatment period.

Body weight and feed consumption: Body weights were recorded daily during treatment period. Feed consumption was determined weekly.

Vaginal Opening

Beginning on PND 22, female pups were examined daily for vaginal opening. The appearance of a small pin hole, a vaginal thread and complete vaginal opening was recorded on the day they were observed. The appearance of a small pin hole or vaginal thread, was recorded until complete vaginal opening observed. The age and weight of female pups were recorded on first day of complete vaginal

Table 3: Estrous Cycle Data

Group N° -with Dose	G1 – 0 mg/kg	G2 – 100 mg CNB/kg	G3 -100 mg QNL/kg	G4 – 150/200 mg/QNL/kg
Cycle status on day of terminal sacrifice				
Diestrus	10	5	4	3
Proestrus	2	-	3	-
Estrus	2	-	3	1
Not cycling	1	1	1	-
Mean Age at First Vaginal Estrus (PND)	39.17	39.00	39.20	39.67

Table 4: Serum Thyroid Hormone levels

Group N° -with Dose	G1 – 0 mg/kg		G2 – 100 mg CNB/kg		G3 -100 mg QNL/kg		G4 – 150/200 mg/QNL/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T3 (ng/mL)	0.995	0.165	0.817*	0.213	0.754**	0.155	0.778**	0.175
TSH (ng/mL)	0.821	0.189	0.933	0.289	0.849	0.172	0.860	0.194
T4 (µg/dL)	2.372	0.199	2.224	0.232	2.246	0.219	2.349	0.121

Note: * = Means different from control at $p<0.05$, ** = Means different from control at $p<0.01$.

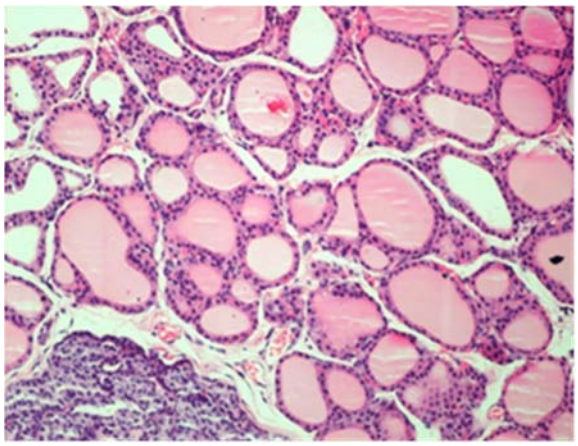

Figure 4A: Thyroid from control group (G1): showing normal sized follicles. (H & E. 200X)

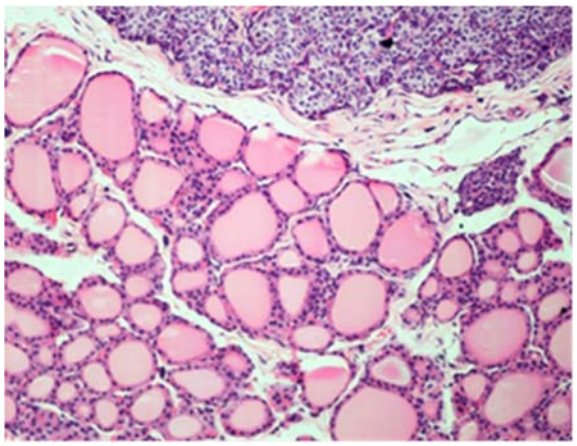

Figure 4B: Thyroid from QNL treated at 150/200 mg/kg; showing normal sized follicles comparable to control (H & E. 200X)

opening. The observations were made approximately at the same time each day.

Estrous Cyclicity

Vaginal smear was observed from the day of vaginal opening, through to and including the day of necropsy. The smear was classified into proestrus, estrus, metestrus and diestrus and the stage was recorded daily[13]. Age of the female was recorded at first vaginal estrus observed[14]. The vaginal smear was observed at approximately the same time each day.

Blood Collection

During necropsy, 2 mL blood from the trunk of the pups was collected immediately after decapitation in serum separation tubes. The collected blood was kept at room temperature, at least for 15 minutes before being centrifuged. Blood samples were centrifuged at 2500-3000 rpm for 15 minutes. The serum was pipetted into micro centrifuge tubes (2 aliquots were prepared) and stored at -80 ± 5°C for subsequent evaluation of hormones.

Blood Chemistry Analysis

The standard clinical chemistry parameters were evaluated including Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Creatinine, Glucose, Alkaline Phsophastase (ALP), and Urea.

Thyroid Hormone Analysis

Blood from the trunk was collected from all surviving animals on day of terminal sacrifice for estimation of serum thyroid hormone level. Serum hormone level analysis was performed using enzyme-linked immunosorbent assay (ELISA). All samples were run in duplicate including QC samples. Kit user guide was used for analysis.

Pathology

The post-mortem examination was performed on F_1 generation female pups, which died during the study after weighing. The endpoints like organ weight, clinical pathology, a histopathology, and hormone analysis was not

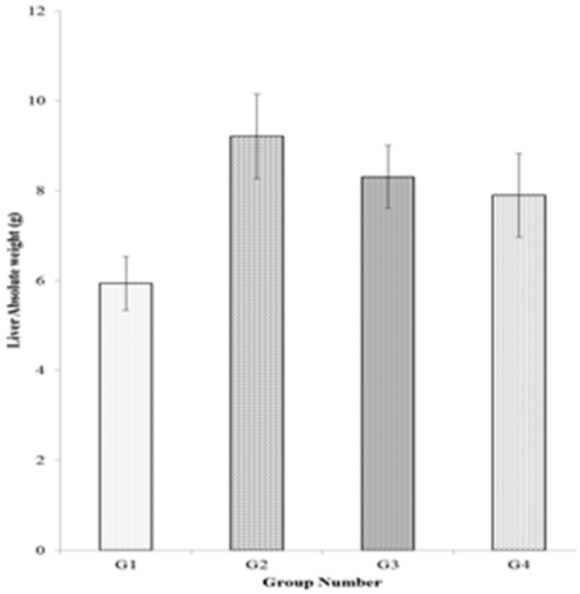

Figure 5A: Liver absolute weight (g)

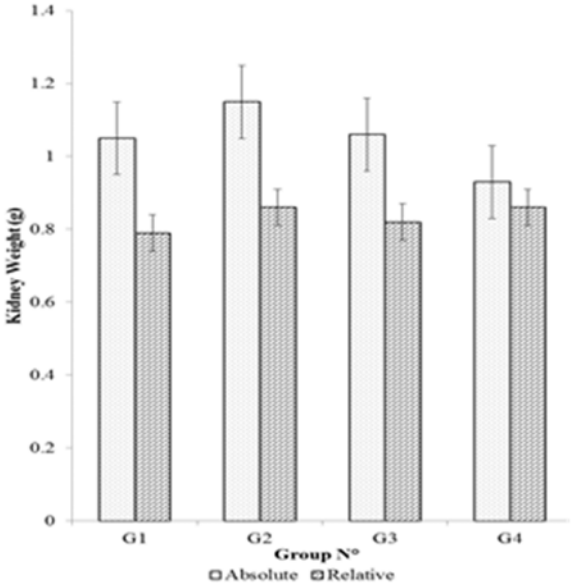

Figure 5B: Kidney weight (g)

carried out for these animals. On PND 41, i.e., one day before the terminal sacrifice, F_1 generation female pups were transferred to a holding room. On PND 42, i.e., on day of terminal sacrifice, dose administration was performed in holding room. The sacrifice of female pups begun 2 hours after dose administration and necropsy was completed by 1 pm. The female pups were sacrificed by decapitation, after administrating injectable anesthetic (sodium thiopentone). The order of necropsy was randomized across all groups being necropsied that day. The complete necropsy examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal, and pelvic cavities, including viscera was performed.

Organ Weight and Collection: At necropsy, ovaries (without oviduct), uterus (wet and blotted), thyroid (with parathyroid), liver, kidneys, pituitary and adrenal were excised, weighed and preserved for subsequent histopathology.

Microscopy Examination

The uterus, thyroid, one ovary, one kidney, liver, pituitary, and adrenal were evaluated for pathological abnormalities and potential treatment related effects. The fixed tissues were processed routinely and stained with hematoxylin and eosin (H & E) for subsequent histological evaluations. Thyroid sections were evaluated for follicular cell height and colloid area using a five-point grading scale (1 = shortest/smallest; 5 = tallest/largest). A minimum of two sections of each of the two lobes of the thyroid were evaluated. Ovarian histology following H & E staining includes an evaluation of follicular development (including presence/absence of tertiary/antral follicles, presence/absence of corpora lutea, changes in corpus luteum development and in number of both primary and atretic follicles) in addition to any abnormalities/lesions. Five random sections were evaluated using the method of Smith, B.J. *et al*[15].

Statistical Analysis

All endpoints were analyzed for heterogeneity of variance using Bartlett test. In the event of a homogeneous data, a parametric one-way analysis of variance (ANOVA) was used to determine intergroup differences. When ANOVA revealed significant intergroup variance, Dunnett's test was used to compare the test item treated groups to the control group. Methods of transformation were applied to heterogeneous data. When transformed data were heterogeneous, the data were analyzed by the Kruskal-Wallis nonparametric ANOVA test. Wherever the results of this ANOVA were statistically significant, Dunn's test was applied to the data to compare all test item treated groups to the control group. The age at partial and complete vaginal opening, body weight at vaginal opening, initial body weight (PND 22), final body weight (PND 42), body weight gain (PND 42-22), and all organ weights were also analyzed by analysis of covariance (ANCOVA) using the body weight at PND 21 as the covariate[16]. Wherever the ANCOVA revealed significant intergroup variance, "t" test was used to compare each test item treated group to the control group at 5% and 1% level of significance.

RESULT

Dose Range Finding Study

Terminal body weight and feed consumption was significantly decreased at 200 mg/kg. Approximately 9% body weight gain was decreased in animals given QNL at 200 mg/kg as compared to the control group. All other endpoints (like clinical chemistry and organ weight) of all dose groups including high dose were comparable with concurrent the control group. Based on the results of the study, 100 and 200 mg/kg were selected for *in vivo* endocrine disruptor studies.

Female Pubertal Assay Result

Observation

No mortality was observed in the groups treated with corn

Table 5: Absolute Organ Weights

Organs	G1 – 0 mg/kg		G2 – 100 mg CNB/kg		G3 -100 mg QNL/kg		G4 – 150/200 mg/QNL/kg	
(Absolute weight)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals (mg)	40.28	4.78	36.78	4.81	43.11	4.64	36.36	4.62
Ovaries (mg)	39.05	8.57	39.35	12.06	39.47	7.35	29.45*	8.63
Uterus (wet) (mg)	211.17	95.77	162.62	57.8	173.51	75.05	110.80**	52.67
Uterus (blotted) (mg)	180.77	60.6	150.75	54.45	161.07	72.33	96.02**	41.51
Thyroid (mg)	13.65	2.46	15.75	2.61	15.38	3.15	15.33	2.83
Liver (g)	5.94	0.6	9.21**	0.94	8.31**	0.70	7.90**	0.93
Kidney (g)	1.05	0.1	1.15*	0.1	1.06	0.08	0.93**	0.14
Pituitary (mg)	6.43	1.82	5.47	1.87	6.81	2.74	4.55*	1.26

Note: * = Means different from control at $p<0.05$, ** = Means different from control at $p<0.01$

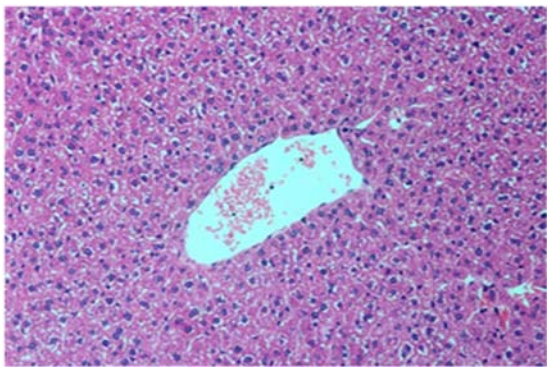

Figure 6A: Liver from control group (G1): showing normal sized hepatocytes around central vein (Centrilobular). (H & E. 200X)

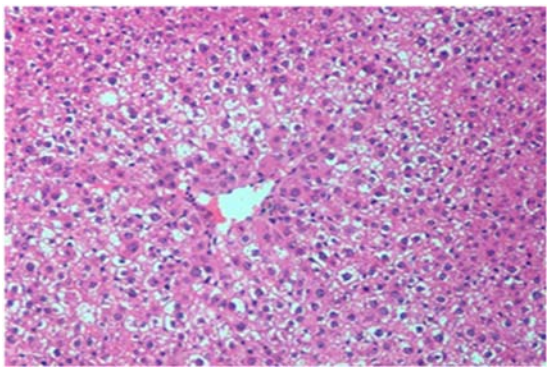

Figure 6B: Liver from QNL treated at 150/200 mg/kg: showing increased size of hepatocytes (Hypertrophy) around central vein (Centrilobular). (H & E. 200X)

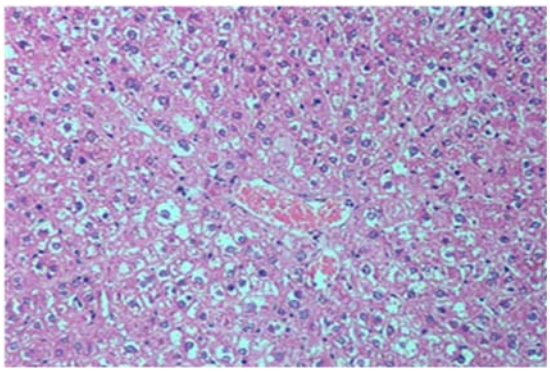

Figure 6C: Liver from CNB treated group: showing increased size of hepatocytes (Hypertrophy) around central vein (Centrilobular). (H & E. 200X)

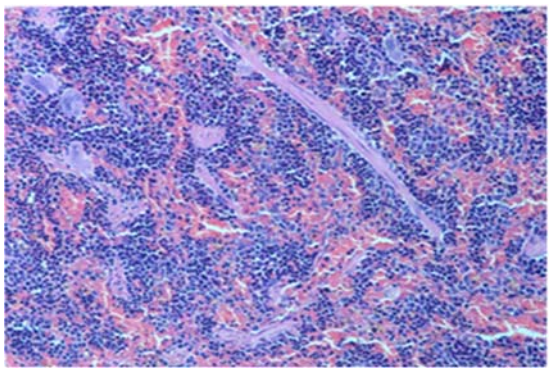

Figure 7: Spleen from CNB treated group: showing moderate extramedullary hematopoiesis (H & E. 200X)

oil, CNB, or low dose of QNL. Two mortalities were observed in high dose of QNL (200 mg/kg) on postnatal day 25. Based on the severity of observation and treatment duration, high dose was reduced to 150 mg QNL/kg. There were no clinical signs observed in rats treated with corn oil, CNB and QNL at 100 mg/kg. Lethargy was observed in all rats treated with QNL at 150/200 mg/kg from PND 24 to PND 31. Two rats treated with QNL at 200 mg/kg had weakness, before death. All other animals appeared to be normal as the treatment progressed till the end of treatment period.

Body Weight and Body Weight Gain

A significant reduction in body weight from PND 23 to 29 was observed in animals treated with CNB at 100 mg/kg; however, it was considered as a transient to treatment as body weight was recovered towards the end of treatment, when compared with the control group. Significant reduction in body weight from PND 23 to 36 was observed in animals treated with QNL at 100 mg/kg; however, body weight was comparable towards the end of treatment period, with the control group. Statistically significant reduction in animal body weight from PND 23 to 42 was observed in rats treated with QNL at 150/200 mg/kg when compared with the control group (Table 1). No significant

difference was observed in body weight gain in animals treated with CNB and QNL at 100 mg/kg. Significant reduction in body weight gain during PND 22 – 42 was observed in the animals treated with QNL at 150/200 mg/kg (Figure 2).

Feed Consumption

A significant decrease in feed consumption was observed from PND 22 to 29 at 100 mg/kg body weight/day of CNB and QNL groups when compared to control group; however, towards end of treatment period feed consumption was comparable. Feed consumption was significantly lower throughout the treatment period (PND 22 to 29, 29 to 36, and 36 to 42 intervals) in the rats treated with QNL at 150/200 mg/kg body weight/day. The decrease in feed consumption during the treatment period correlated with the reduction in body weight and body weight gain in the respective dose group (Figure 3).

Vaginal Opening

There were ten (100 mg/kg body weight/day of CNB), four (100 mg/kg of QNL) and eleven (150/200 mg/kg of QNL) female rats which did not attain complete vaginal opening till PND 42. Mean age at incomplete vaginal opening, mean age at vaginal opening, body weight at vaginal opening was comparable in animals treated with CNB and QNL at 100 mg/kg as compared to the control group. Mean age at incomplete vaginal opening and mean age at complete vaginal opening was delayed statistically in animals treated with QNL at 150/200 mg/kg as compared to the control group. Body weight at vaginal opening was significantly decreased in rats treated with QNL at 150/200 mg/kg (Table 2).

Estrous Cyclicity

Animals treated with QNL at 100 mg/kg had no effect on estrous cyclicity. Animals treated with CNB at 100 mg/kg and with QNL at 150/200 mg/kg showed irregular cycling. Mean age at first vaginal estrous was comparable for all treated groups as compared to the control group; however, considerable delay in onset of vaginal opening was observed in animals treated with CNB at 100 mg/kg and with QNL at 150/200 mg/kg which precluded proper evaluation of the estrous cycle and mean age at first vaginal estrous (Table 3).

Thyroid Hormone

Serum T3 level was significantly decreased in CNB and QNL treated groups as compared to control group. Serum thyroid levels of QNL and CNB treated groups were comparable with the concurrent control group (Table 4). It was also supported microscopic examination where no evidence of treatment related change in thyroid follicles was observed (Figure 4A and 4B).

Clinical Chemistry

No treatment related difference was observed in QNL and CNB treated groups in various parameters of clinical chemistry.

Organ Weight

Organ weight of liver (unadjusted, adjusted and relative) was significantly increased in QNL treated groups (Figure 5A). Values of the unadjusted and adjusted organ weight for kidneys were significantly decreased while relative weight was significantly increased in group treated with QNL at 150/200 mg/kg as compared to the control group (Figure 5B). Organ weight of pituitary (unadjusted) was significantly decreased in group treated with QNL at 150/200 mg/kg. Besides an increase in relative weight in pituitary, was observed in all QNL treated groups. Values of the unadjusted and adjusted organ weight of uterus (both wet and blotted) and ovary were significantly decreased in group treated with QNL at 150/200 mg/kg as compared to the control group (Table 5). In CNB treated groups, relative organ weight of liver, kidney, pituitary, and adrenals was significantly increased as compared to the control group. Organ weight of liver (Unadjusted and adjusted) and kidneys (adjusted) was significantly increased as compared to the control group (Table 5).

Follicular Cell Height and Colloid Area of Thyroid

Result of evaluation of follicular cell height and colloid area of thyroid did not reveal treatment related difference between the control group and high dose group (Figure 4A and 4B).

Follicular Count in Ovaries

Statistical analysis of follicular count, growing as well as antral follicles in ovaries was significantly reduced in group treated with QNL at 150/200 mg/kg. Animals treated with QNL at 100 mg/kg had comparable follicular count with the control group.

Microscopic Findings

Microscopic examination of liver revealed centrilobular hypertrophy of hepatocytes in 12/13 rats treated with QNL at 150/200 mg/kg and 15/15 rats in the groups treated with CNB at 100 mg/kg (Figure 6A, 6B, and 6C). Animals treated with CNB at 100 mg/kg had enlarged spleen. All animals showed extramedullary hematopoiesis (EMH) in red pulp of spleen (Figure 7).

DISCUSSION

The EDSP Tier 1 Endocrine Screening Battery is intended to be a suite of *in vitro* and *in vivo* assays to identify the potential of a chemical to interact with the estrogen, androgen, thyroid, or steroidogenesis systems[17]. While evaluating Tier 1 data of *in vivo* and *in vitro* assay for potential endocrine disruptor activity, weight of evidence (WoE) should be considered for its testing in Tier 2. Within the context of the current EDSP Tier 1 battery, results of *in vitro* assays alone generally would not be expected to provide a sufficient basis to support the need for Tier 2 testing. When weighing the different lines of evidence and examining the balance of positive and negative results, EPA expects that *in vivo* evidence would typically be given greater overall influence in the WoE evaluation than *in vitro* findings because of the inherent limitations of such assays, which include the inability to account for absorption, distribution, metabolism, and excretion of the compound, as well as normal intact physiological conditions (e.g., the ability of an animal to compensate for endocrine alterations). The relative sensitivity and specificity of the measured endpoints would also be relevant considerations[18].

Dose Level Selection and Clinical Sign

Based on the results of dose range finding study, high dose - 200 mg/kg body weight/day was selected with the aim

that it would be established as maximum tolerated dose. In dose range finding, animals of age 6 – 7 weeks were treated for 14 days, terminal body weight loss was 9% and lethargy was observed on first two days of dosing, however as the treatment progressed animals become accustomed with QNL treatment at 200 mg/kg. Whereas in present study, animals were treated from postnatal day 22 for 20 days, terminal body weight loss was 24% and animals had lethargy for 7 – 8 days of post-dosing. As two animals belonging to high dose of QNL died on 4th day of treatment, dose was reduced to 150 mg/kg. Results reveal that age of the animals plays a crucial role for setting dose for endocrine disruptor studies. In this particular study, maximum tolerated dose was exceeded as the body weight was significantly reduced in animals treated with 150/200 mg/kg body weight.

Body Weight and Feed Consumption

Animals treated with QNL at 100 mg/kg body weight had 3% decrease in terminal body weight, though the animals had significant decrease in body weight, from postnatal day 23 to 36. Body weight gain was decreased initially compared to control group animals. However, as the treatment progressed, body weight gain was increased compared to previous day body weight of respective animal and towards the end of treatment period animals had minimal (3%) decrease in body weight which was also supported by comparable feed consumption towards the end of treatment which reveals that as the treatment progressed animals become accustomed to its treatment. Animals treated with QNL at 150/200 mg/kg body weight had marked reduction in body weight and feed consumption which reveals that QNL had produced the systemic toxicity in animals. Animals had 24% decrease in body weight compared to control group. Presence of sign of toxicity (i.e., lethargy) for 7 days of post-dosing reveals that the maximum tolerated dose (MTD) has been achieved. The MTD for body weight is defined as the dose that produces a 10% reduction in terminal body weight as compared with the appropriate control group[19,20].

Vaginal Opening

Mean age at incomplete vaginal opening and mean age at complete vaginal opening (VO) was delayed by more than 1.3 and 1.5 days respectively in animals treated with CNB at 100 mg/kg body weight/day as compared to control group. Mean age at incomplete vaginal opening and mean age at complete vaginal opening was delayed by more than 1 and 0.9 days respectively in animals treated with QNL at 100 mg/kg body weight/day as compared to control group. Mean age at incomplete vaginal opening and mean age at complete vaginal opening was delayed statistically by more than 4 and 2 days respectively in animals treated with QNL at 150/200 mg/kg body weight/day as compared to control group. The animals treated with QNL at 100 mg/kg showed 3-4% decrease in body weight and body weight at puberty was decreased by 2-3%. Mean age at puberty onset was delayed by not more than 0.9 days. However, animals treated with QNL at 150/200 mg/kg showed 25-26% decrease in body weight at VO, and mean age at VO was delayed by 4 days. Similar findings were observed by Stoker[21]; Marty[22]. The interpretation of delays in puberty onset in concurrence with decreased body weight (> 10% body weight, exceed MTD) is an extremely common problem for reproductive toxicologist. This is related to the fact that puberty onset is an apical end point which is not only influenced by sex steroid hormones, but also by body weight, body composition, and other general factors[23,24]. An evaluation of the impact of decrease in body weight on puberty onset indicated that it is greatly dependent on the intensity and magnitude of decrease in body weight[25]. Many endpoints included in the pubertal assays can be altered by changes in rate of growth and/or terminal body weight, making it difficult to interpret assay data and discern specific endocrine-mediated effects. There are some conflicting reports on the sensitivity of puberty onset to moderate changes in body weight/growth rate. Laws *et al*[16] reported that 20–21% decrease in body weight did not significantly affect age at puberty onset in male or female rats, suggesting age at puberty onset is insensitive to changes in growth. However, other studies suggest that age at puberty onset and body weight function as a continuum[26], and body weight alterations of approximately 10–15% could alter puberty onset in male rats[21,25]. The differences reported in these publications may be related to the rate at which the body weight decrement occurred (i.e., how quickly it occurred and over what time frame/ages). Whether this effect is secondary to a decrease in growth rate or systemic toxicity remains to be determined. However, the combined weight of evidence from these studies suggests that body weight reductions of approximately 20% at the time of puberty onset may represent an important transition point at which body weight can have a large impact on mean age of puberty onset (i.e., delays of several days).

Estrous Cyclicity

Mean age at first vaginal estrous stage was comparable for animals treated with QNL at 100 and 150/200 mg/kg body weight as compared to control group. Despite the fact that considerable delay in onset of vaginal opening was observed at 100 mg CNB/kg and 150/200 mg QNL/kg, which ultimately precluded proper evaluation of the estrous cycle and mean age at first vaginal estrous. The mean day of attainment of vaginal patency typically occurs a few days before the necropsy of the females on PND 42. Normal estrous cycle length in rats is 4–5 days. If monitoring begins mid-cycle, it may take 8 days or longer to observe two estrus stages to determine estrous cycle length. The test guideline requires each female to be characterized as “regularly cycling,” “irregularly cycling,” or “not cycling”. However, the monitoring interval may not allow for the evaluation of a full estrous cycle, particularly if an animal is slightly older at the time of vaginal opening. In these cases, it may not be possible to determine if an animal is cycling normally, because the monitoring period is too short. In addition, there are inter animal differences in the duration of estrous cycle monitoring such that monitoring across the dose groups is often inequitable. It is not uncommon for young animals to cycle abnormally with the initiation of estrous cycling (it usually takes until about 8 weeks of age for normal cycles to occur consistently) and the estrous cycle also can be infl

uenced by other factors such as stress and feed intake[27,28,29]. To perform a thorough assessment of estrous cyclicity, there is a need of 2–3-week period as required in the other reproduction study protocol. Hence for this study, QNL effect on estrous cycle remains unanswered.

Clinical Chemistry and Thyroid Hormone

In the present study, animals treated with QNL at 100 and 150/200 mg/kg showed comparable clinical chemistry parameters and thyroid hormone levels (i.e., T3, T4 and TSH) with concurrent control group. And it was also confirmed by histopathological evaluation as no microscopic changes were observed in the thyroid. Thus, it appears that the QNL has no effect on female thyroid which is also supported by comparable thyroid weight.

Organ Weight and Microscopic Examination

In the present study, increase in liver weight was observed in the QNL treated animals. QNL, at 150/200 mg/kg produced histopathological features centrilobular hepatocyte hypertrophy which could be considered due to CYP450 induction. However, exact mechanism is unknown. Previously it was noted that centrilobular hepatocellular hypertrophy was associated with increases in absolute liver weights of >20%. However, there was no relationship between the magnitude of liver weight increase or hepatocellular hypertrophy and the degree of CYP450 induction. Importantly, CYP450 induction, hepatocellular hypertrophy and increased absolute liver weights, in the absence of other histologic findings, were not associated with changes in serum ALT activity or other measured serum hepatic enzymes[30]. Significant decrease in kidney and pituitary weight was observed in animals given QNL at 150/200 mg/kg. Whereas kidney and pituitary weight of QNL treated animals at 100 mg/kg was comparable with control group. In microscopic examination, no microscopic lesion effect on kidney was observed. Hence, this could be considered to secondary effect to significant decrease in body weight. Uterus (both blotted and wet) and ovary weight were significantly decreased as compared to control animals given QNL at 150/200 mg/kg. In microscopic examination a significant reduction in growing, as well as antral follicles, was observed in animals treated with QNL at 150/200 mg/kg. Whereas, no effect was seen in primordial follicles and in animals treated with QNL at 100 mg/kg. Effect on large growing or antral follicles may cause interruption of cyclicity. This effect is generally reversible, if exposure to the toxicant ceases. More follicles can ultimately, be recruited for development from the pool of primordial follicles that remains unaffected[31].

CONCLUSION

In summary, the results of the present experiment indicate that reductions in body weight up to 24% in peripubertal female rats showed considerable delay in puberty, despite the fact that whether this effect is secondary to systemic toxicity or it is an effect of endocrine disruption is unknown. Quinoline had no effect on thyroid. Lower dose of Quinoline (100 mg/kg) showed no sign of systemic toxicity and pubertal delay.

CONFLICT OF INTEREST

Author declares there is no conflict of interest.

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ABBREVIATIONS

AAALAC = Association for Assessment and Accreditation of Laboratory Animal Care International, CNB = 1-Chloro-2-Nitrobenzene, EDSTAC = Endocrine Disruptor Screening and Testing Advisory Committee, EDSP = Endocrine Disruptor Screening Program, FQPA = Food Quality Protection Act, HPV = High Production Volume, H & E = Haematoxylin and Eosin Stain, IAEC = Institutional Animals Ethics Committee, LO(A)EL = Low Observed (Adverse) Effect Level, MOA = Modes of Action, MTD = Maximum Tolerated Dose, NO(A)EL = No Observed (Adverse) Effect Level, PND = Postnatal Day, QNL = Quinoline, T3 = Triiodothyronine, T4 = Thyroxine, TSH = Thyroid Stimulation Hormone.

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