Effect of Polycyclic Aromatic Hydrocarbon Ingestion on Japanese Quail Chicks

For this study, investigators evaluated the toxicological effects in the quail chicks following oral exposure to naphthalene and determined threshold doses at which these effects occur. The results of this study will provide an important tool for predicting long-term injuries to avian species from oil spills.

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Background:

Petroleum products contain alkylated naphthalen and phenanthrene

PAHs are primary persistent chemicals from oil spills

Their toxicity profile in birds is not characterized – limiting toxicity assessment

Why Japanese Quail?

Standard bioassay model specie

Well known husbandry parameters

Quick life cycle

Readily available

Summary of Previous Work

Started on experimental diets at 6 wks of age (near adult)

Treatment 1: Control
Treatment 2: 50 ppm naphthalene
Treatment 3: 100 ppm naphthalene
Treatment 4: 200 ppm naphthalene

Collect eggs during the 11th week, incubate, feed chicks
0 naphthalene for 2 wks

After 12 weeks, adults were killed and tissues collected

Summary:
Parameter significantly affected by Naphthalene

Final weight	200 ppm
Weight gain	200 ppm
Feed intake	200 ppm
Kidney weight (female)	100 ppm ?
Hematocrit	200 ppm
Intestinal histology	200 ppm

No evidence for major reproductive effects

(Egg number, fertility, hatchability, chick viability)

Protocol and Experimental Design

200 Quail hatched (July 30) – 168 closest in body weight and lacking health problems were chosen for the study

Distributed randomly to 24 pen with 7 chicks per pen,

3 pens assigned to each treatment group

Treatment 1: Control
Treatment 2: 50 ppm naphthalene
Treatment 3: 100 ppm naphthalene
Treatment 4: 200 ppm naphthalene
Treatment 5: 400 ppm naphthalene
Treatment 6: 800 ppm naphthalene

Birds and feeders weighed on days 0, 3, 6, 10 & 14

On day 14:

- Birds bled (blood pooled within a pen)
- Birds killed with CO_2
- Tissues collected

Lab analysis:

- Hematology
- Acute phase proteins
- Clinical chemistries
- Gross Pathology (No signs)
- Histopathology

Statistics - ANOVA and student-t tests using SAS JMP v.7.

RESULTS:

Effect of Naphthalene on Gain, Feed Intake & Efficiency day 14

Level	Gain	Intake	Efficiency
0	45.7	93.3	0.49
50	46.0	90.1	0.51
100	46.0	92.0	0.5
200	46.5	94.9	0.49
400	45.8	91.6	0.5
800	45.3	92.5	0.49
SEM	1.4	3.6	0.03
P value	0.89	0.85	0.79

Effect of Naphthalene on organ weights (g)

Level	Liver	Bursa	Spleen	Kidney
0	1.22	0.044	0.040	0.053
50	1.36	0.045	0.039	0.066
100	1.22	0.050	0.037	0.066
200	1.15	0.047	0.042	0.067
400	1.16	0.042	0.035	0.055
800	1.14	0.041	0.031	0.058
SEM	0.08	0.005	0.0030	0.006
P value	0.41	0.79	0.17	0.41

Effect of Naphthalene on organ weights (g/100 g BW)

Level	Liver	Bursa	Spleen	Kidney
0	2.66	0.09	0.087	0.12
50	3.00	0.10	0.086	0.14
100	2.66	0.11	0.082	0.14
200	2.48	0.10	0.091	0.14
400	2.53	0.09	0.076	0.12
800	2.53	0.09	0.068	0.11
SEM	0.19	0.009	0.006	0.017
P value	0.48	0.77	0.13	0.52

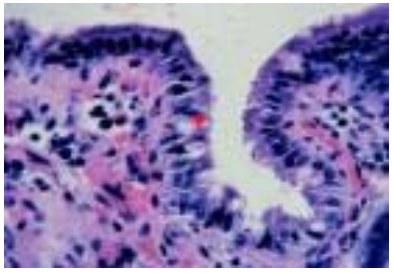
Level	Hematocrit (%)	Hemoglobin (g/dl)
0	36.7^{AB}	6.5^{B}
50	37.3^{AB}	6.4^{BC}
100	37.0^{AB}	6.6^{B}
200	35.7^{BC}	6.0^{AB}
400	38.0^{A}	5.5^{A}
800	34.3^{C}	5.9^{AC}
SEM	0.64	0.2
P value	0.02	0.03

Effect of Naphthalene on WBCs (#/100)

Level	Hetero	Lympho	Mono	Eosin	Baso
0	44.3^{B}	39.0^{A}	7.0	6.3	3.3
50	44.3^{B}	40.3^{A}	5.7	5.3	4.3
100	57.0^{A}	31.7^{AB}	4.7	3.7	3.0
200	64.7^{A}	17.3^{C}	6.3	7.3	4.3
400	65.3^{A}	20.3^{BC}	4.7	7.3	2.3
800	53.7^{AB}	32.0^{A}	5.7	5.3	3.3
SEM	4.0	3.8	1.74	1.4	1.1
P value	0.008	0.004	0.91	0.48	0.8

Effect of Naphthalene on Intestinal Histology

Treatment	lamina propria (µm)	villus height (µm)	villus width (µm)	crypt depth (µm)	intra-epithelial lymphocytes (#/villi)	lamina propria leukocytes (#/villi)
0	$22^{\Lambda B}$	221^{C}	37	45	$11^{\Lambda B}$	21^{Λ}
50	$23^{\Lambda B}$	215^{C}	35	43	9^{Λ}	$25^{\Lambda B}$
100	$26^{\Lambda B}$	233^{C}	34	45	$11^{\Lambda B}$	20^{Λ}
200	20^{Λ}	220^{C}	39	47	$16^{\Lambda B}$	$31^{\Lambda B}$
400	$24^{\Lambda B}$	$195^{\Lambda B}$	33	41	$16^{\Lambda B}$	28^{Λ}
800	30^{B}	180^{Λ}	39	39	21^{B}	39^{B}
SEM	2.1	7.8	3.1	2.9	3.1	4.2
P value	0.05	0.02	0.41	0.11	0.04	0.04



No Significant Treatment Differences in:

Serum protein (g/dl)	3.7 ± 0.2
Tryglycerides (mg/L)	79 ± 6.6
ALT (IU/L)	12.8 ± 2.0
LD (IU/L)	422 ± 21
AST (mg/L)	501 ± 28
Uric acid (mg/L)	8.5 ± 1.3
Albumin (mg/L)	1.9 ± 0.4
Haptoglobin (ug/dl)	2.4 ± 0.3
Lysozyme (mg/ml)	13.3 ± 2.5

Summary:
Parameter significantly affected by Naphthalene

Hematocrit	800 ppm
Hemoglobin	400 ppm
Blood Heterophils	100 ppm
Blood Lymphocytes	200 ppm
Villi height	400 ppm
Lamina propria leukocytes	800 ppm

Reproductive study – Some indication at 100 ppm
Solid indication at 200 ppm

Growth study – some indication at 100 ppm