Evaluation of the toxic effects of eight environmental chemicals in adult Japanese Quail (*Coturnix japonica*)

Emily Boulanger [1], Amani Farhat[2], YeonSeon Jeon[1], Niladri Basu[1], Markus Hecker [3], Doug Crump [2], Jessica Head[1]

1-Faculty of Agriculture and Environmental Sciences, McGill University, Montreal, Quebec, Canada; 2-National Wildlife Research Centre, Environment and Climate Change Canada, Ottawa, Ontario, Canada; 3- Toxicology Centre, University Saskatchewan, Saskatoon, Saskatchewan, Canada

Introduction

Background:

- Ecological and regulatory risk assessments depend on toxicity data for a wide range of taxonomic groups
- Toxicity reference values (TRVs) for birds are typically provided by standardized, adult avian toxicity tests
- Key standardized avian toxicity tests include: OECD223 (Avian Acute Oral Toxicity Test) and the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OCSPP Number 850.2100
- For the present study, adult Japanese quail toxicity studies were conducted by a research team at Eurofins EAG Agroscience, LLC following OCSPP Number 850.2100

Objectives:

- Determine and characterize apical outcomes in adult Japanese quail exposed to 8 chemicals commonly detected in Canadian ecosystems: benzo[a]pyrene (BaP); lead (II) nitrate (Pb); seleno-L-methionine (SeMe); hexabromocyclododecane (HBCD); ethinylestradiol (EE2); fluoxetine hydrochloride (FLX); trenbolone (TB) and chlorpyrifos (CPF)
- Quantify liver concentrations of administered chemicals at 2 distinct timepoints
- Contribute data to the EcoToxChip project: a toxicogenomics tool for chemical prioritization and environmental management (www.ecotoxchip.ca)

Table 1. Concentrations of 8 environmental chemicals in dosing solutions and liver tissue from adult JQ toxicity tests

	DOSE	CHEMICALS							
		EE2	CPF	TB	BaP	SeMe	Pb	FLX	HBCD
Nominal Dosing Concentration (ppm)	S	Corn Oil							
	LD	0.05	0.1	0.1	0.5	0.1	35	1	10
	MD	0.5	1	1	5	1	350	10	100
	HD	5	10	10	50	10	3500	100	1000
Liver Concentration Day 4 (ppm)	S	<MLOD	<MLOD	<MLOD	<MLOD	0.64	0.03	<MLOD	<MLOD
	LD	<MLOD	<MLOD	<MLOD	<MLOD	0.76	0.34	<MLOD	0.006 $\gamma^{\diamond}$
	MD	<MLOD	<MLOD	<MLOD	<MLOD	1.1	6.54	<MLOD	0.02 α; 0.04 γ
	HD	<MLOD	<MLOD	<MLOD	<MLOD	6.64	-	0.04	0.09 α; 0.02 γ
Liver Concentration Day 14 (ppm)	S	<MLOD	<MLOD	<MLOD	<MLOD	0.82	0.04	<MLOD	<MLOD
	LD	<MLOD	<MLOD	<MLOD	<MLOD	0.60	0.17	<MLOD	<MLOD
	MD	<MLOD	<MLOD	<MLOD	<MLOD	1.2	0.86	<MLOD	0.01 α; 0.01 γ
	HD	<MLOD	<MLOD	<MLOD	<MLOD	2.15	-	<MLOD	0.04 α; 0.02 γ

S = solvent, LD = low dose, MD = medium dose, HD = high dose, and MLOD = method limit of detection

$^{\diamond}$Two different stereoisomers of HBCD were detected in liver tissue, γ-HBCD is the predominant isomer in the technical mixture used in the present study.

Experimental Design

Three studies were carried out at Eurofins EAG Agroscience, LLC: **study 1:** EE2, CPF and TB (December 2017); **study 2:** BaP and SeMe (July 2018); and **study 3:** Pb, FLX and HBCD (October 2017)

- In each study, chemicals were tested at 3 doses plus a shared solvent control (i.e. corn oil); the high dose was designed to cause ≤20% mortality
- Birds were obtained at one or two days of age from Loudounberry Farm & Garden, Leesburg, VA (in each study, all birds were from the same hatch)
- Prior to chemical exposure, birds were acclimated to the facility for 7 – 12 weeks (which included a test pen acclimation for the last 4 – 9 weeks)
- Birds were fasted for 16.5 – 18 h prior to dosing
- On the first day of the experiment, a single dose (4 mL/kg of body weight) of the test substance in corn oil was orally administered by gavage (the birds were 7 – 12 weeks old when exposed)
- Each dose group included a total of 20 Japanese quail (10 males + 10 females) that were randomly assigned to test pens
- During the experiment, birds were maintained at ambient room temperature (**study 1:** average temperature was 17.6°C; **study 2:** average temperature was 22.5°C; and **study 3:** average temperature was 21.9°C)
- A light/dark cycle of 8 h/16 h was maintained during acclimation and the experiment (fluorescent lighting which was approximated to the color spectrum of noonday sunlight)
- From test initiation (Day 0) until termination (Day 14), all birds were observed at least twice a day (mortalities, signs of toxicity, and abnormal behaviors were recorded)
- Body weight of each bird was measured on Day 0, 4, 7, and 14 of the experiment
- Average daily feed consumption was evaluated by pen over three distinct time periods: Day 0-4, Day 4-7, and Day 7-14
- Throughout acclimation and testing, birds were fed a game ration ad libitum (except during fasting prior to and following (1-2 h) dosing)
- On Day 4, 6 birds per dose group (3 per sex) were euthanized with CO_2 and liver (studies 1-3) and gonads (study 1 only) were collected, weighed and stored for future analysis (i.e. omics and chemical residue analysis)
- On Day 14 (termination), up to 14 birds per dose (7 per sex) were euthanized with CO_2 and liver (studies 1-3) and gonads (study 1 only) were collected, weighed and stored for future analysis (i.e. omics, chemical residue analysis and histology)
- Gross necropsies were performed on all dead birds and those that survived to Day 4 and Day 14. This included examination of the exterior of the bird and thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system

Gavage into the crop

ENDPOINTS (Day 4) : N=6
- Apical Outcomes (mortality, body weight, abnormalities, liver and gonad weights)
- Omics (transcriptomics, metabolomics, and proteomics)
- Chemical Residue

ENDPOINTS (Day 14) : N=14
- Apical Outcomes (mortality, body weight, abnormalities, liver and gonad weights)
- Transcriptomics
- Chemical Residue
- Histology

Results

Table 2a. Japanese quail mortality and morphometrics following oral administration of chemicals by gavage. Results are presented as the average value for all individuals in each group. See table 2b for values that were significantly different when males and females were analyzed separately.

Category	Exposure Day	EE2				CPF			TB			BaP				SeMe			Pb				FLX			HBCD		
		0 mg/kg	0.05 mg/kg	0.5 mg/kg	5 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0 mg/kg	35 mg/kg	350 mg/kg	3500 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg	10 mg/kg	100 mg/kg	1000 mg/kg
Mortality	Day 4 [n=20]	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0	20	0	0	0	0	0	0
	Day 14 [n=14]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0	0
* Mean Body Weight (g)	Day 0 [n=20]	305 (±38)	297 (±22)	299 (±32)	302 (±26)	299 (±26)	299 (±26)	309 (±26)	322 (±40)	303 (±27)	306 (±28)	300 (±23)	290 (±29)	288 (±30)	286 (±25)	304 (±26)	300 (±26)	301 (±22)	275 (±22)	259 (±24)	266 (±25)	256 (±18)	256 (±15)	266 (±18)	264 (±20)	271 (±18)	261 (±16)	254 (±17)
	Day 4 [n=20]	312 (±38)	305 (±20)	307 (±32)	308 (±30)	303 (±28)	304 (±27)	313 (±25)	329 (±44)	311 (±24)	315 (±28)	307 (±20)	304 (±29)	305 (±30)	304 (±27)	320 (±28)	315 (±26)	295 (±35)	289 (±26)	272 (±25)	275 (±24)	–	275 (±16)	282 (±18)	274 (±20)	293 (±17)	278 (±17)	273 (±17)
	Day 7 [n=14]	315 (±32)	320 (±19)	317 (±36)	326 (±32)	321 (±31)	319 (±29)	320 (±25)	337 (±50)	317 (±22)	319 (±31)	312 (±26)	305 (±38)	303 (±31)	311 (±27)	320 (±31)	327 (±29)	315 (±28)	306 (±24)	285 (±24)	289 (±27)	–	280 (±13)	293 (±21)	285 (±21)	298 (±19)	285 (±16)	284 (±24)
	Day 14 [n=14]	315 (±37)	323 (±21)	314 (±37)	327 (±34)	322 (±27)	322 (±31)	324 (±25)	343 (±51)	320 (±21)	328 (±27)	324 (±33)	311 (±38)	312 (±38)	319 (±31)	321 (±45)	342 (±30)	331 (±24)	305 (±25)	284 (±28)	295 (±29)	–	281 (±16)	300 (±22)	291 (±22)	300 (±20)	296 (±17)	283 (±23)
*Mean Feed Consumption (g/bird/day)	Day 0 - 4 [n=20]	36	38	43	35	36	38.5	39.5	46.5	37.5	38.5	35.5	44.5	65	61	52	50.5	49	34.5	35	33.5	–	38.5	36	36.5	37	42.5	38
	Day 4 - 7 [n=14]	47	42	49	45	54.5	66	66	62	60	47.5	53	37	81	75	86	66	71.5	44	39	47	–	48	44.5	43.5	39	42	43.5
	Day 7 - 14 [n=14]	40	35	42	41	44.5	51.5	50.5	51.5	50.5	43.5	54.5	34.5	56	54.5	59.5	48	56	39.5	34.5	41	–	42.5	37	30	37	36	33.5
* Mean Liver Weight (g)	Day 4 [n=6]	5.6 (±1.3)	4.8 (±0.9)	5.4 (±0.4)	5.9 (±0.9)	5.0 (±1.5)	4.6 (±1.2)	4.9 (±0.5)	5.8 (±0.5)	6.0 (±0.7)	6.1 (±1.0)	5.0 (±0.5)	5.8 (±1.3)	6.2 (±0.4)	6.4 (±1.1)	6.4 (±0.5)	6.2 (±0.3)	7.0 (±0.6)	7.0 (±0.8)	6.7 (±1.1)	6.8 (±0.9)	–	6.6 (±1.4)	6.0 (±0.2)	6.7 (±0.5)	6.9 (±0.4)	5.9 (±0.9)	5.8 (±1.2)
	Day 14 [n=14]	5.7 (±1.0)	5.8 (±1.4)	5.3 (±0.8)	6.1 (±1.2)	6.0 (±0.6)	6.8 (±0.9)	6.2 (±0.9)	6.7 (±0.9)	6.3 (±1.2)	6.6 (±1.2)	5.9 (±0.8)	6.1 (±1.3)	6.2 (±1.3)	6.2 (±1.2)	5.9 (±1.3)	6.4 (±1.4)	5.9 (±0.8)	6.5 (±0.6)	6.5 (±0.7)	7.0 (±1.2)	–	5.9 (±0.5)	6.7 (±0.8)	6.7 (±0.8)	6.9 (±1.0)	6.9 (±0.9)	6.4 (±0.6)

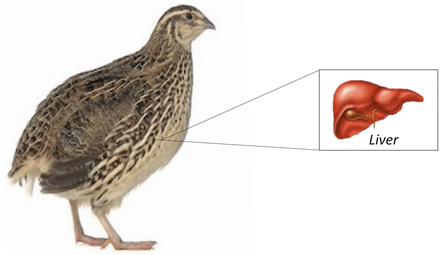
*The data presented in the body weight and liver weight categories represent the mean value followed by the standard deviation in parentheses (±).
"n=" represents the sample size per dose; ; "–" = No morphometric data available for Pb high dose due to mortality

°Mean daily feed consumption was determined by pen

Table 2b. Morphometrics separated by sex. Data are only shown for chemicals where separation by sex led to significant effects.

Category	Exposure Day	Sex	EE2				CPF			BaP				SeMe		
			0 mg/kg	0.05 mg/kg	0.5 mg/kg	5 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg
* Mean Body Weight (g)	Day 0 [n=10]	M	285 (±33)	293 (±20)	291 (±26)	**301 (±24)**	293 (±31)	290 (±26)	301 (±24)	NE	NE	NE	NE	302 (±28)	296 (±25)	**303 (±28)**
		F	325 (±34)	302 (±23)	306 (±37)	**304 (±29)**	305 (±20)	308 (±24)	**318 (±28)**	NE	NE	NE	NE	306 (±26)	304 (±27)	**298 (±14)**
	Day 4 [n=10]	M	293 (±32)	301 (±19)	300 (±25)	**299 (±27)**	295 (±34)	293 (±28)	307 (±22)	NE	NE	NE	NE	322 (±32)	310 (±29)	**302 (±41)**
		F	332 (±35)	308 (±20)	313 (±38)	**318 (±31)**	311 (±19)	315 (±22)	**318 (±27)**	NE	NE	NE	NE	317 (±24)	319 (±23)	**291 (±32)**
	Day 7 [n=7]	M	301 (±31)	321 (±17)	310 (±25)	321 (±31)	314 (±34)	305 (±29)	315 (±22)	NE	NE	NE	NE	323 (±37)	319 (±31)	330 (±32)
		F	329 (±28)	319 (±22)	325 (±45)	330 (±34)	327 (±28)	333 (±24)	325 (±29)	NE	NE	NE	NE	317 (±25)	336 (±27)	308 (±26)
	Day 14 [n=7]	M	296 (±34)	319 (±17)	304 (±28)	322 (±35)	318 (±33)	309 (±30)	319 (±25)	NE	NE	NE	NE	310 (±56)	331 (±26)	346 (±30)
		F	334 (±30)	328 (±25)	324 (±44)	331 (±35)	326 (±25)	335 (±27)	329 (±25)	NE	NE	NE	NE	332 (±29)	352 (±32)	325 (±20)
* Mean Liver Somatic Index (%)	Day 4 [n=3]	M	NE	NE	NE	NE	NE	NE	NE	1.6 (±0.2)	1.7 (±0.1)	2.0 (±0.4)	2.0 (±0.2)	**2.0 (±0.2)**	**2.1 (±0.2)**	**2.5 (±0.5)**
		F	NE	NE	NE	NE	NE	NE	NE	1.6 (±0.2)	2.1 (±0.4)	1.9 (±0.2)	**2.3 (±0.4)**	1.9 (±0.2)	**2.0 (±0.1)**	**2.5 (±0.3)**
	Day 14 [n=7]	M	NE	NE	NE	NE	NE	NE	NE	1.8 (±0.2)	2.0 (±0.4)	1.9 (±0.3)	1.9 (±0.2)	1.6 (±0.3)	1.7 (±0.2)	1.7 (±0.1)
		F	NE	NE	NE	NE	NE	NE	NE	1.8 (±0.2)	1.9 (±0.4)	**2.1 (±0.2)**	2.0 (±0.5)	2.0 (±0.3)	2.0 (±0.4)	1.8 (±0.2)

Values in **bold/blue** font were significantly different from the control
"n=" represents the sample size per dose per sex ; NE = no significant effect observed ; M = male and F = female



Results

Table 3. Signs of toxicity and gross necropsy observations

Chemical	Dose	Signs of Toxicity	@Gross Necropsy Observations (on Day 4 and 14)
BaP	5 mg/kg	–	On Day 4: one pale spleen
	50 mg/kg	–	On Day 4: one pale kidneys, one pale liver and one retained yolk sac. On Day 14: one thin with a prominent keel, enlarged spleen and pasty cecal contents
SeMe	0.1 mg/kg	–	On Day 14: three head lesions and one foot lesion
	1 mg/kg	(slight) ruffled appearance (50% of birds)	–
	10 mg/kg	ruffled appearance, lethargy, wing droop, and shallow and rapid respiration (80% of birds)	Dead Birds (n=4): all 4 had a cooked appearance of the breast muscle. 3/4 with gelatinous material around crop, pale liver, and empty gastro-intestinal tract. 2/4 with a mottled liver and one bird looked thin, had a beige fluid in crop and dark fluid in gastro-intestinal tract. On Day 4: two pale livers, one thin with a loss of muscle mass and slightly prominent keel, and a small heart and spleen.
Pb	0 mg/kg	–	On Day 3: one severe head lesion and was euthanized. On Day 4: one with bruising and a lesion on the head.
	35 mg/kg	–	On Day 14: one with foot lesions
	3500 mg/kg	ruffled appearance, wing droop, lethargy, lower limb weakness, prostrate posture, loss of coordination, loss of righting reflex, and depression (100% of birds)	Dead Birds (n=20): 11/20 had whitish material in the crop, 11/20 had tissue surrounding the crop whitish in color, 6/20 had empty gastrointestinal tract (GI), 4/20 had GI tract containing whitish material or greenish/brownish fluid, 1/20 had loss of muscle mass, 5/20 had a pale pancreas, 1/20 had a pale spleen and 2/20 had pale kidneys.
FLX	100 mg/kg	ruffled appearance, wing droop, lethargy, lower limb weakness, prostrate posture, loss of coordination, loss of righting reflex, depression and convulsions (100% of birds)	On Day 4: one with foot lesions
HBCD	100 mg/kg	–	On Day 14: one with foot lesions
	1000 mg/kg	ruffled appearance and wing droop (15% of birds)	–

@Head and foot lesions were not considered to be related to treatment.

Table 4. NOEL (no observed effect level) and LOEL (lowest observed effect level)

Chemical	NOEL (mg/kg)	LOEL (mg/kg)	Source of LOEL
EE2	0.5	5	Table 2b
CPF	1	10	Table 2b
TB	>10	–	No effects at any dose
BaP	0.5	5	Table 2b
SeMe	<0.1	0.1	Table 2b
Pb	350	3500	Table 3
FLX	10	100	Table 3
HBCD	100	1000	Table 3

Summary:

- Pb caused 100% mortality at a dose of 3500 mg/kg (Table 2a)
- SeMe caused 20% mortality at a dose of 10 mg/kg (Table 2a)
- Significant effects of chemicals on morphometric endpoints were only observed when individuals were separated by sex (Table 2b)
- Signs of toxicity were observed in SeMe, Pb, FLX, and HBCD exposure studies (Table 3)
- Gross effects during necropsy were observed in BaP, SeMe, Pb, and FLX exposure studies (Table 3)
- LOELs were established for seven of the eight chemicals (Table 4)
- Pending/future analyses include omics, histology and comparison of the adult JQ data to the early-life stage exposures (https://doi.org/10.1002/etc.4582)

Acknowledgments

Funders: Genome Canada; Génome Québec; Genome Prairie; the Government of Canada; Environment and Climate Change Canada; Ministère de l' Économie, de la Science et de l'Innovation du Québec; the University of Saskatchewan; and McGill University.
People: Patrick M Hubbard, B.S. and Diana L. Temple, M.S (from Eurofins EAG Agroscience, LLC), Lisa Bidonosti, and Jenny Eng.
Partners: Environment & Climate Change Canada, US Army Corps of Engineers, US EPA, SGS AXYS, Qiagen, and Shell USA.

EcoToxChip Project Overview

GenomeCanada GenomeQuébec GenomePrairie

McGill University of Saskatchewan Environment and Climate Change Canada

www. ecotoxchip.ca