

EDC Effects on Different Organisms: Consequences of Embryonic Exposure Timing is Everything!

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What are Endocrine Disrupting Chemicals (EDCs)?

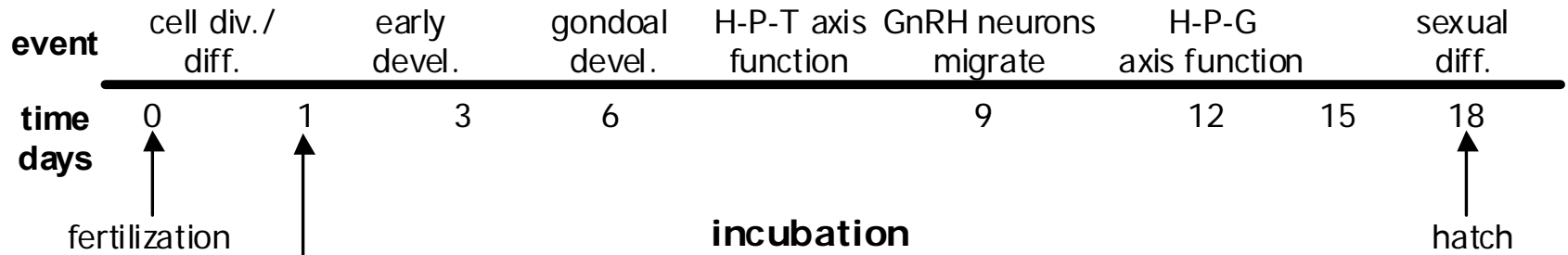
Why do We Care?

- **Biomedical impacts—body burden, maternal transfer**
- **Domestic species**
- **Wildlife damage—individual and population level**
- **Subtle effects—**
 - **Maturation**
 - **Aging**
 - **Disease**
 - **Impaired learning and other implications?**

Today's focus:

- **How do we determine impact?**
 - **Damage to avian species—focus on selected neuroendocrine and behavioral measures**
- **Similarities to mammals—the soy controversy!**

Embryonic Exposure to EDCs in Quail; Timing is Everything!



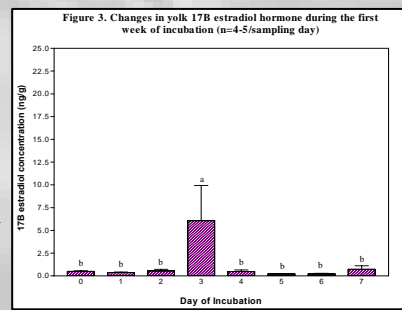
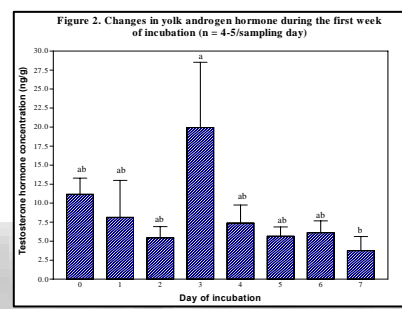
↑
maternal
deposition

↑
impact on
organs and
later function

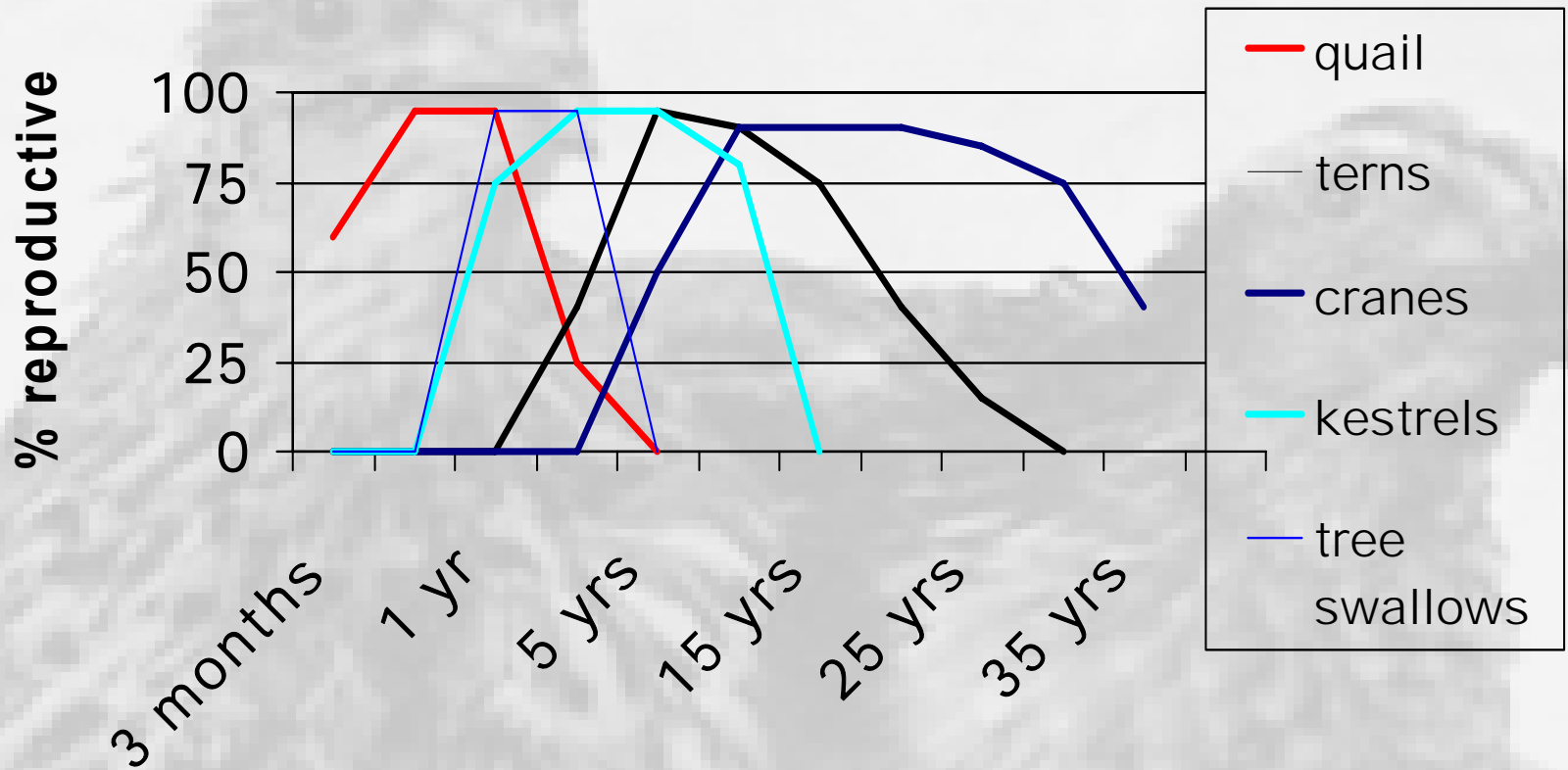
↑
Brain/behavior

↑
T3/T4
Corticosterone

incubation



What about Aging?



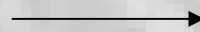
Species	Lifespan (yrs)	Clutch size
Quail	2-5 yrs	6-10
Tree Swallow	3-5 yrs	5-6
Terns	20-30 yrs	2.2
Kestrel	12-15 yrs	4.5
Crane	35-40 yrs	1.4

Assessment and Measurement Endpoints—the so what factor!

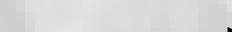
- **Relevance**
- **Short or long term consequences (hatchling vs adult)**
- **Individual variability in response**
- **Stage of life cycle**

- **Transfer to the field for assessment**
- **Potential to have adverse effects for populations**

Laboratory trials



Reproductive impact



Population effects

characterize effects and potential mechanisms of action

consequences of exposure from field observations

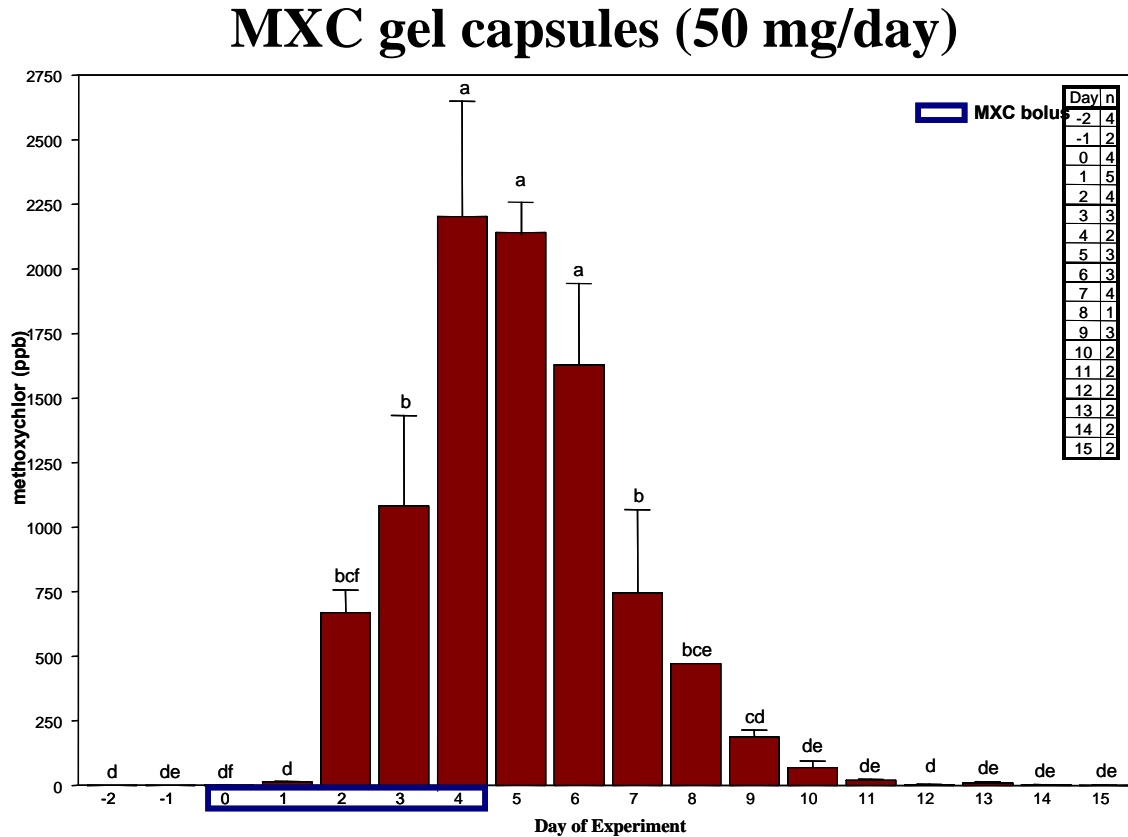
fitness ??

Compounds Considered

- **Estrogenic compounds**
 - **Estradiol (endogenous; pharmacological)**
 - **Diethylstilbestrol (DES; pharmacological)**
 - **Genistein (soy phytoestrogen)**
 - **Methoxychlor (pesticide)**
 - **PCBs (PCB-26, -126, mixture of congeners; industrial)**
- **Androgen active compounds**
 - **Vinclozolin (fungicide)**
 - **DDE (ethylene, 1,1-dichloro-2,2-bis(p-chlorophenyl; Metabolite of DDT)**
 - **Trenbolone acetate (anabolic growth promotent)**
- **Atrazine (herbicide)**
 - **targets aromatase enzyme? Dopamine? GnRH?**

How does Exposure occur in Birds?

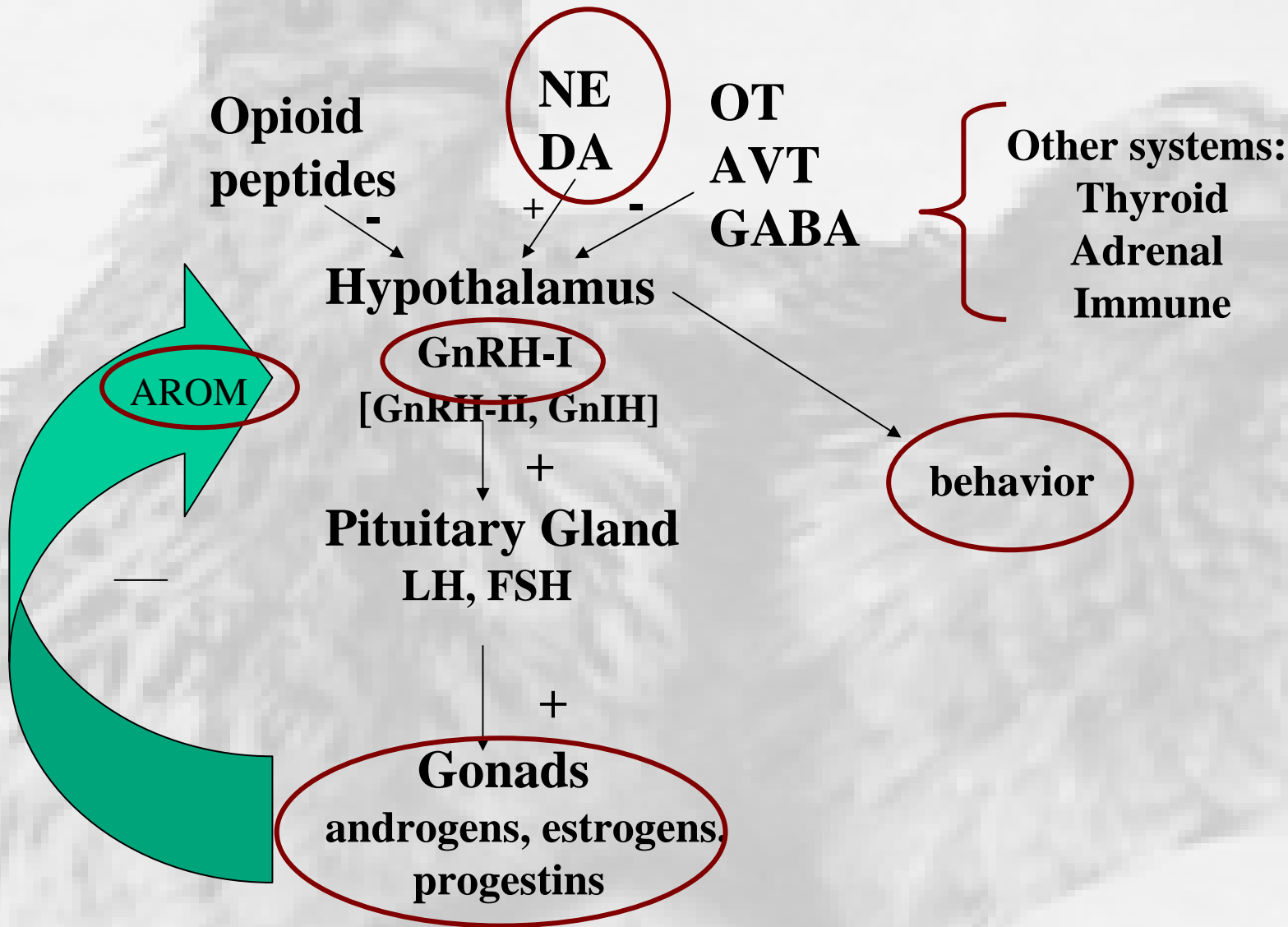
Maternal Transfer



parental exposure → embryonic exposure

Ottinger et al., 2004

What are Likely Targets of EDCs?



Issues:

- Are effects of different classes of EDCs (**and mixtures of EDCs**) similar?
- How do we interpret sublethal and weak effects?
 - What about individual variability (statistics problems)
- What about mixtures—additive or yielding contradictory data?
- How can damage be assessed in avian populations?

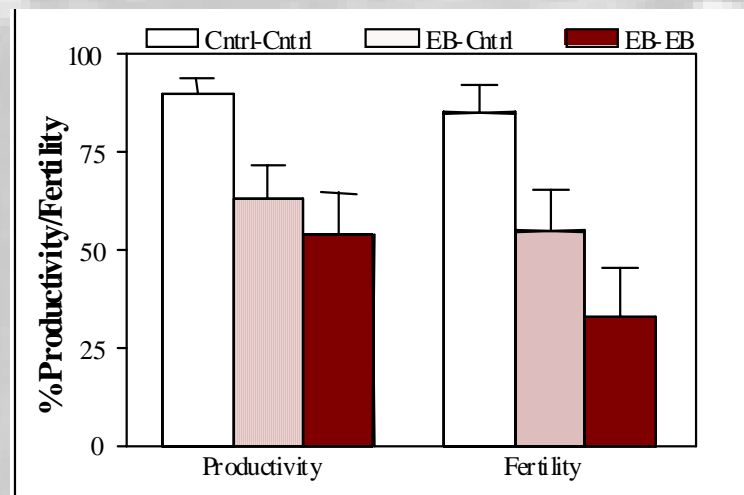


Fertility, Embryo Viability, Growth/Maturation
Classic measures for toxicology

Effects of Injected EDCs: Estradiol

Effects of Embryonic Estradiol Benzoate on Reproductive Parameters (2-generations)

SEX	TRAIT	TREATMENT		
		Control injected	Oil injected	EB
Females	Age at first egg (n=15-30/trt)	53±0.72 ^a	51±0.95 ^a	61±1.7 ^b
	Age at which 20% lay (n=15-34)	56	52	67
	Age at which 50% lay (n=15-34)	60	56	74
	Lay rate (%) (n=14-34)	81 ± 2.5 ^a	81 ± 4.1 ^a	52 ± 8.9 ^b
	Ovary weight (%) (n=14-16)	3.9 ± 0.2 ^a	Not determined	4.0 ± 0.5 ^a
	Body weight (g) (n=14-16)	152 ± 2.6 ^a	Not determined	143 ± 2.6 ^b
Males	Age at cloacal foam production (n=23-33)	49±0.7 ^a	48 ± 1.0 ^a	49 ± 2.0 ^a
	Cloacal gland area (mm ² ; n=6-9)	177±16 ^a	178 ± 13 ^a	172±8 ^a
	Body weight (g)	116 ± 2	115±6	114±2

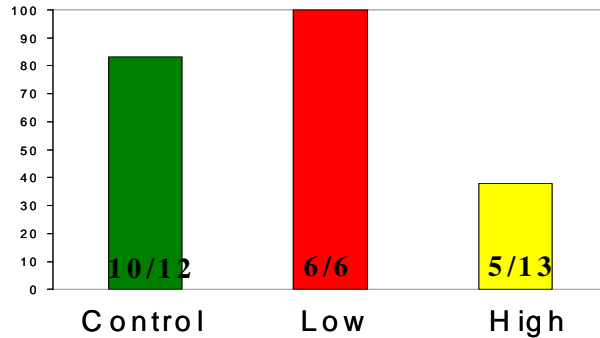


Ottinger, Abdelnabi, Henry, McGary, Thompson, and Wu, Horm. Beh. 40: 234, 2001

University of Maryland March 13, 2007

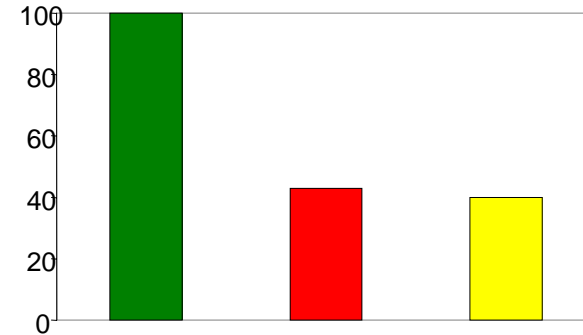
Effects of Dietary Methoxychlor (0.5 and 5ppm)

% F1 Females with Functional Ovaries

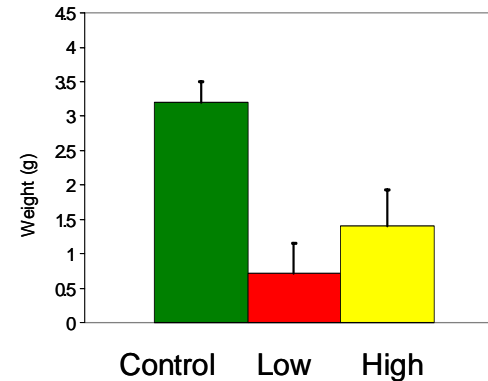


$\chi^2=9.29, p<.05$

% of F2 Males with Developed Testes at 7 Weeks of Age

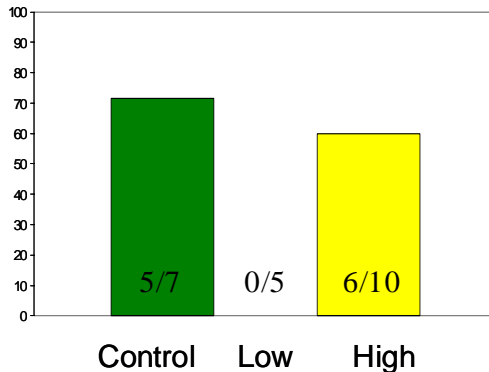


F2 Testes Weight in Males at 7 weeks



Ancova
 $F(2, 13) = 8.03,$
 $p < .05$
(adjusted least square means)

% of F2 Adult Females with Mature Ovaries



$\chi^2=6.69,$
 $p < .05$

Dietary MXC was associated with altered sexual maturation



Male Sexual Behavior

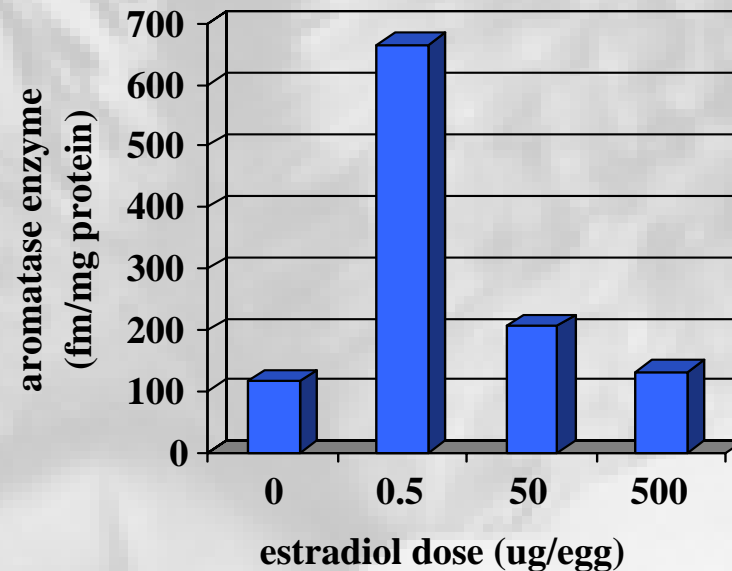
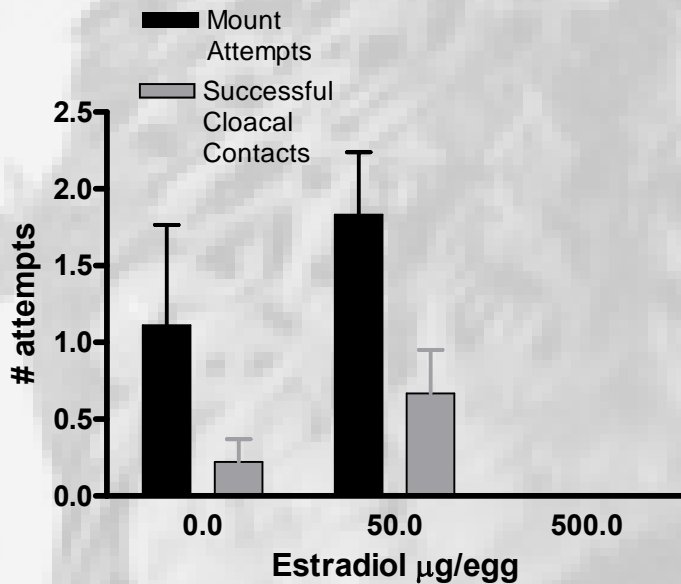
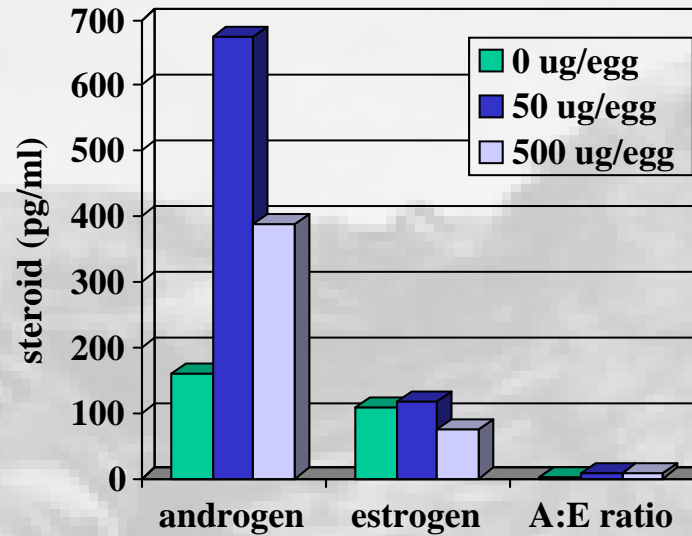
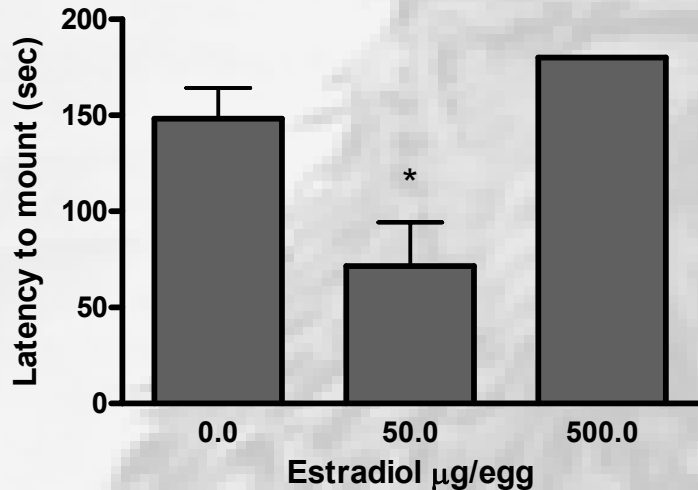
Neurotransmitters and Neuropeptides

Not traditionally considered by toxicology

EDCs—regardless of apparent action
impaired male sexual behavior in quail

- Delayed maturation?
- Permanent effect?
- Long term impact on neural systems
 - Catecholamines
 - Vasotocin (Panzica and Viglietti-Panzica)
 - Aromatase enzyme

Exogenous E₂ at embryonic day 4 or 11 resulted in decreased male behavior, altered steroid levels and aromatase enzyme in adults.



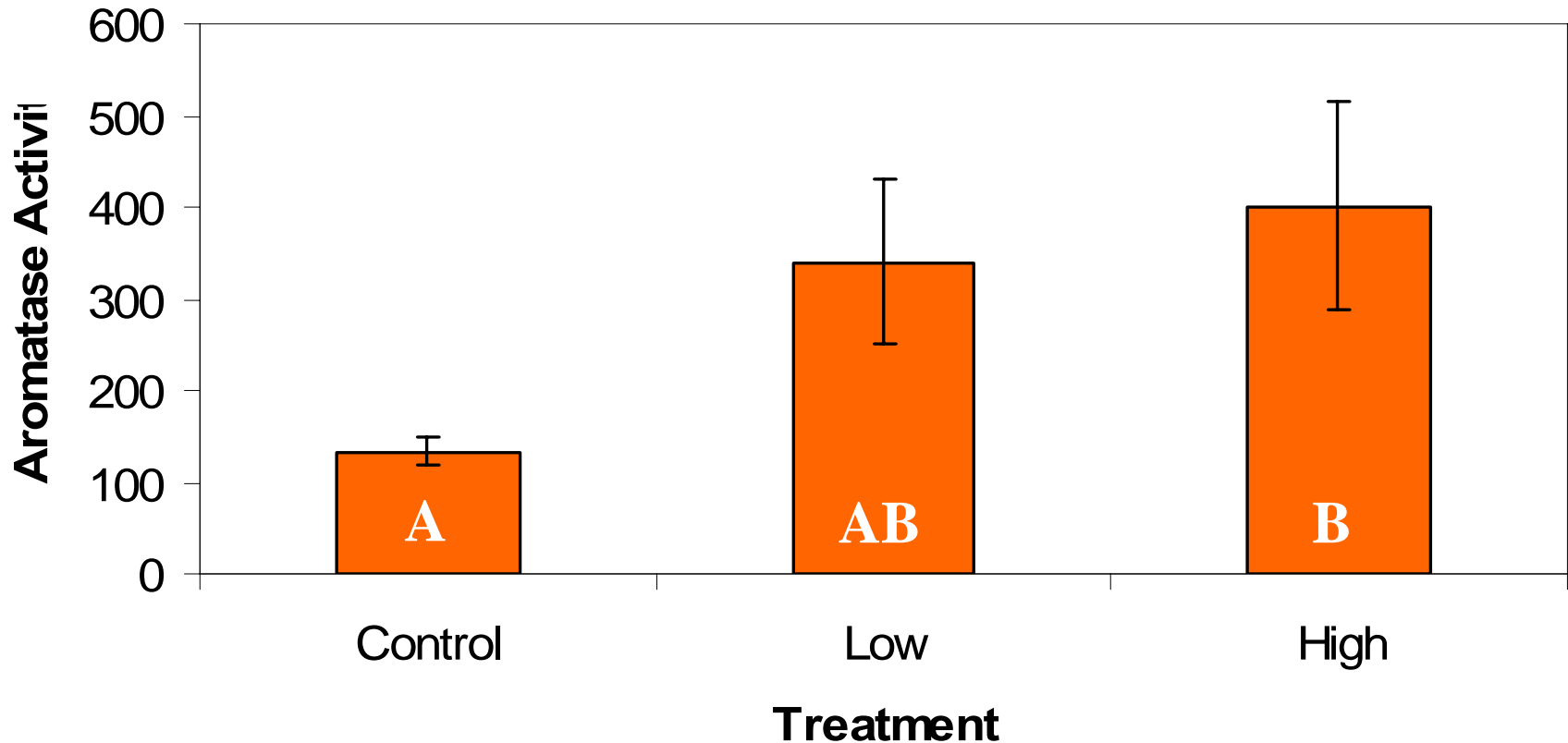
Hypothalamic norepinephrine and dopamine concentrations in adult males that were treated with estradiol benzoate, testosterone propionate, or oil on embryonic days 10, 12, or 14, hatch, or day 7, post hatch (results are expressed as ng/mg protein, $\bar{x} \pm se$; means with different letters differ significantly at the $p < 0.05$ level)

Day of	NE			DA		
Treatment	Control	EB	TP	Control	EB	TP
10	5.0 \pm 0.9 ^a	4.2 \pm 0.5 ^a	5.5 \pm 1.9 ^a	4.3 \pm 1.0 ^a	7.3 \pm 1.2 ^b	3.9 \pm 0.6 ^a
12	10.0 \pm 1.4 ^a	6.0 \pm 1.2 ^b	7.2 \pm 1.7 ^a	3.2 \pm 0.7 ^a	8.5 \pm 1.4 ^b	2.6 \pm 0.2 ^a
14	8.3 \pm 1.2 ^a	7.2 \pm 1.8 ^a	4.4 \pm 0.7 ^a	4.7 \pm 0.9 ^a	4.5 \pm 1.1 ^a	3.7 \pm 0.6
Hatch	7.5 \pm 1.3 ^a	6.6 \pm 0.8 ^a	5.3 \pm 1.4 ^a	5.2 \pm 1.1 ^a	4.2 \pm 0.6 ^a	4.2 \pm 1.6 ^a
7	5.2 \pm 0.3 ^a	9.6 \pm 2.7 ^b	6.6 \pm 0.8 ^a	6.2 \pm 1.5 ^a	4.9 \pm 1.8 ^a	2.8 \pm 0.4 ^a

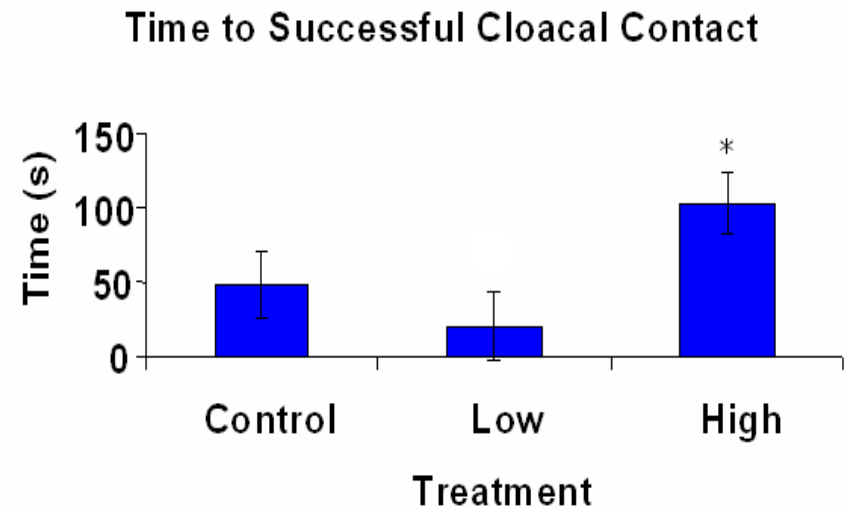
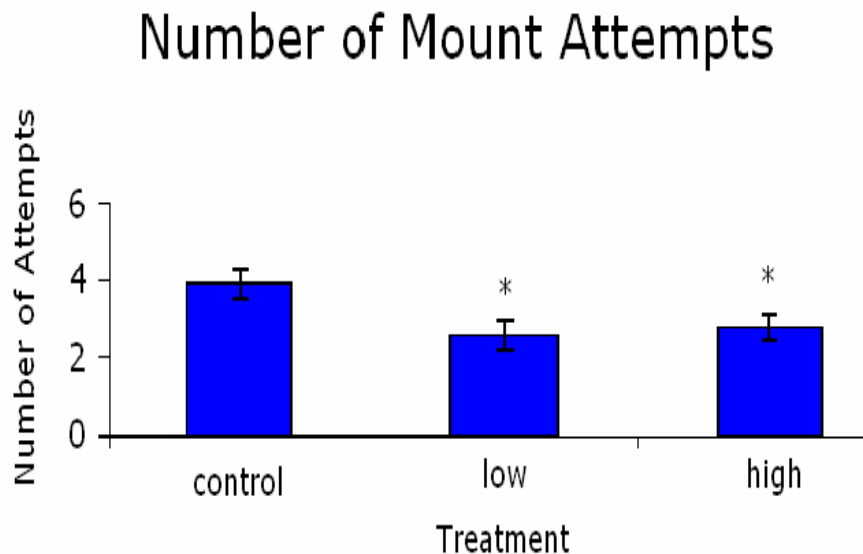
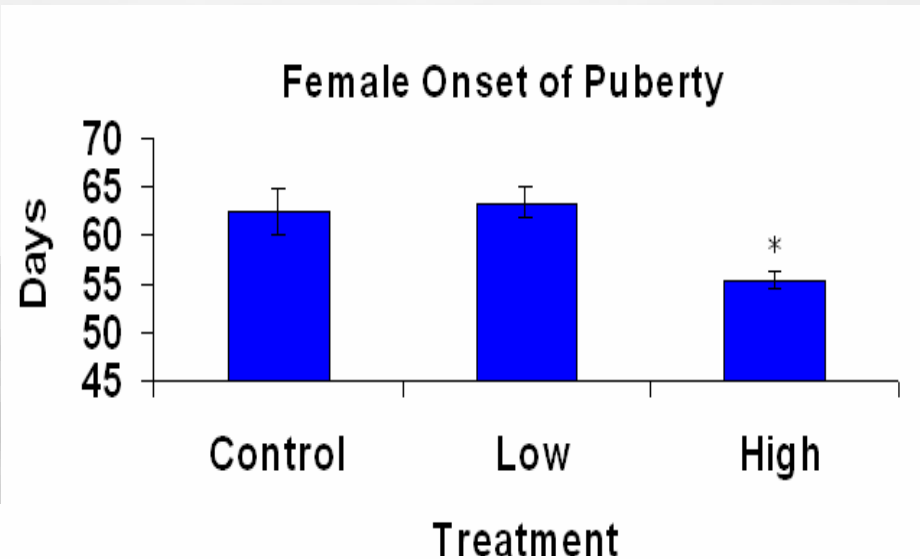
Dietary Methoxychlor (5 and 10ppm) impacted aromatase activity in hatchlings; the 10ppm males showed impaired mating behavior

Aromatase Activity
(fmol/hr*organ)/mg protein

P = 0.0534

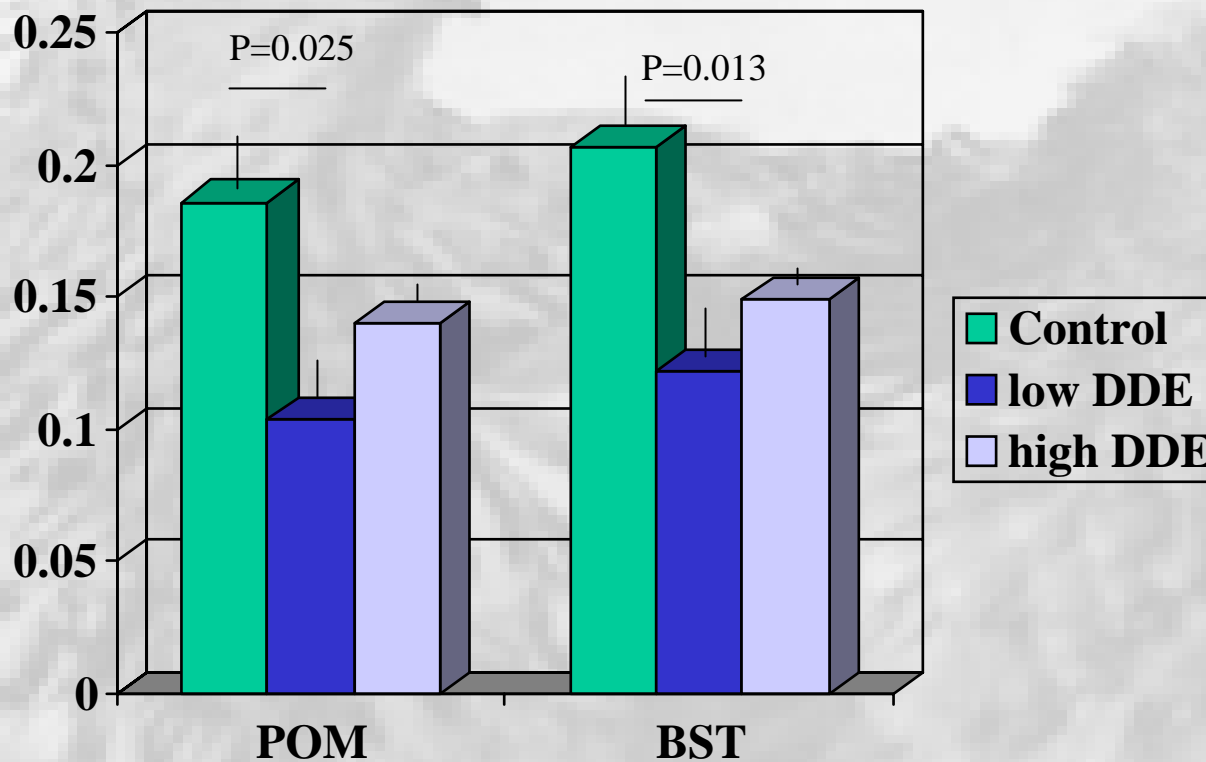


DDE impacted reproductive maturation in females and impaired male mating behavior



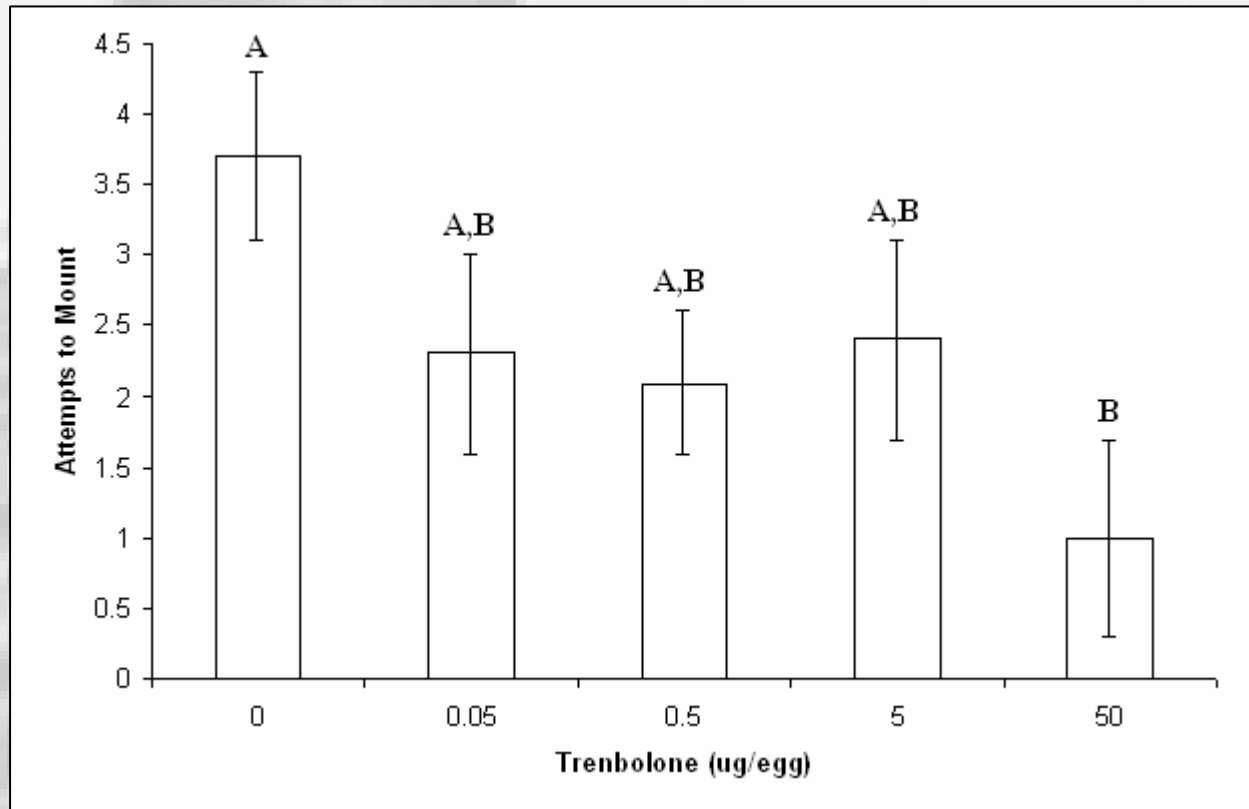
Vasotocin Neurons: Effects of DDE

(Panzica, Quinn et al., 2007)



DDE resulted in significant differences (ANOVA) in the vasotocin immunoreactive neurons in the preoptic nucleus (control vs low) and in the bed nucleus of the stria terminalis (control vs low).

Embryonic exposure to trenbolone acetate impaired male behavior



Number of times male Japanese quail attempted to mount females (mean +/- SEM) in the first of three reproductive behavioral test trials. Significant differences between treatments are indicated by asterisk ($p < 0.05$). Quinn et al., 2006

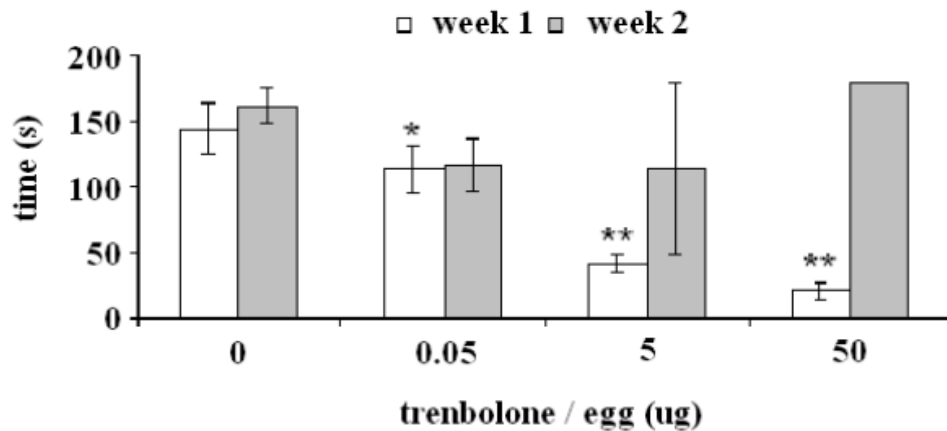


Figure 19. Time required (mean +/- SEM) for isolated Japanese quail chicks to rejoin conspecifics 182 cm away at one and two weeks of age. Significant differences between treatments are indicated by asterisks ($p < 0.01$).

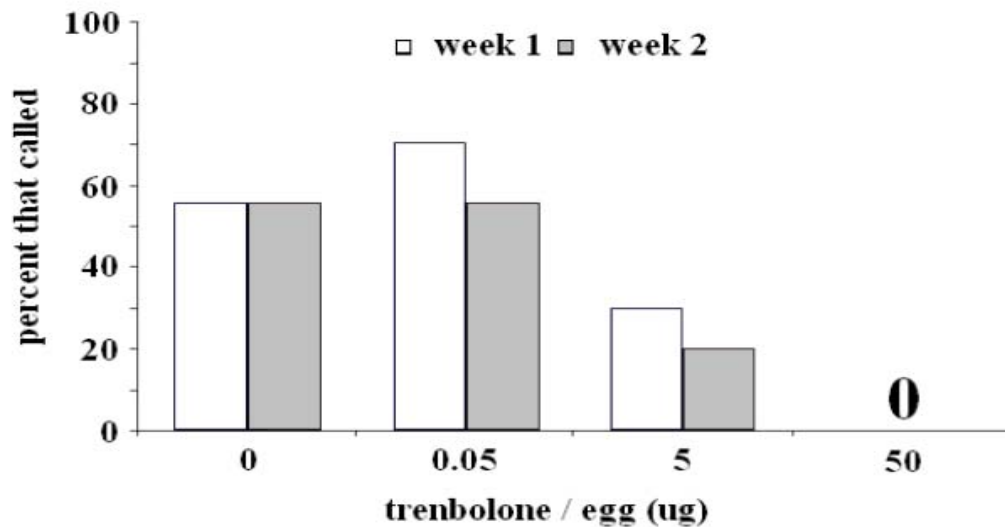


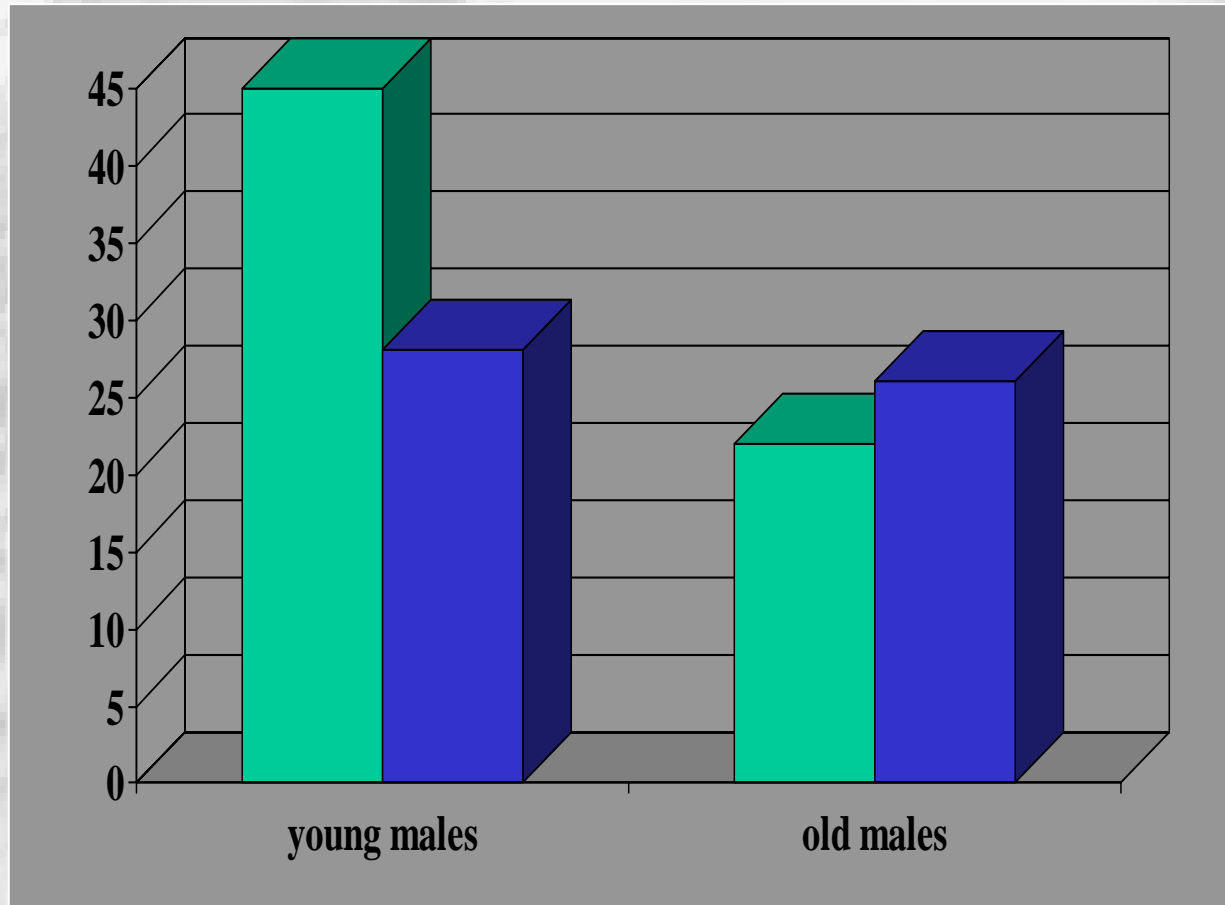
Figure 21. Percent of isolated Japanese quail chicks that called to conspecifics during a three-minute motor behavior test at one and two weeks of age.

Trenbolone also had interesting effects on motor behavior (modified open field test) and on calling (data from Quinn et al., unpublished data).



Gonadotropin Releasing Hormone-I
(GnRH-I)—effects on this system was
surprising due to many redundancies in
neuroendocrine regulation of this system!

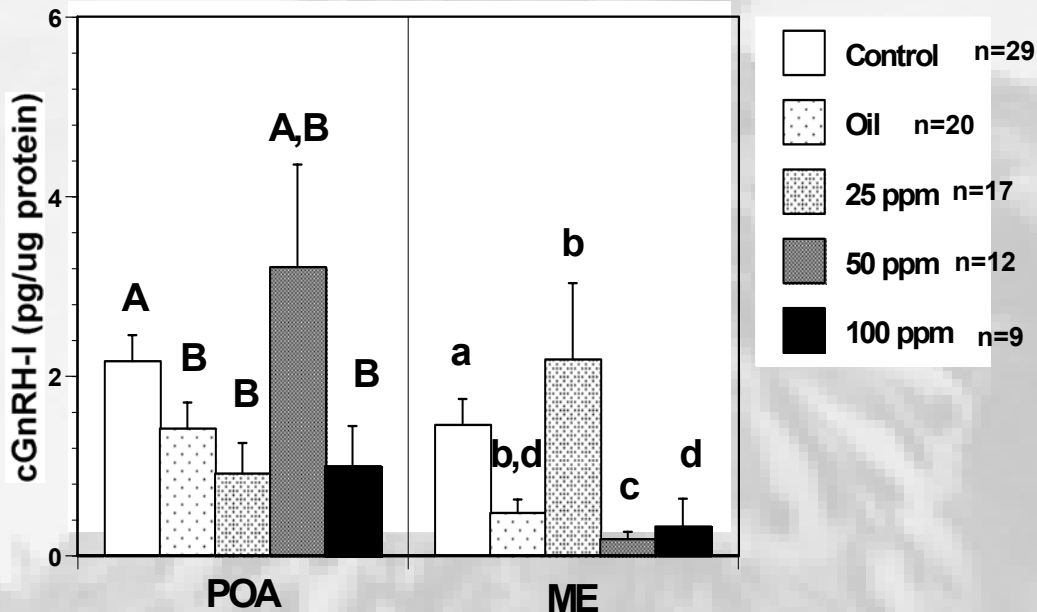
Number of GnRH-I-ir cells (mean/animal) in POA-SL of aging male and female quail is sexually dimorphic and changes with aging.



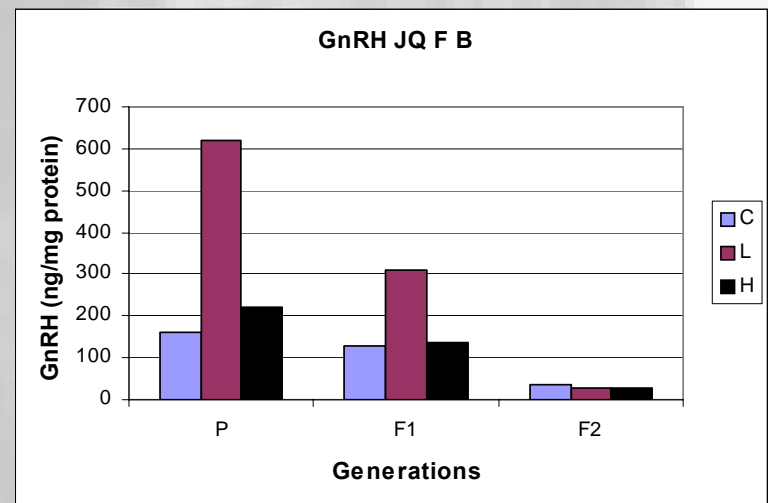
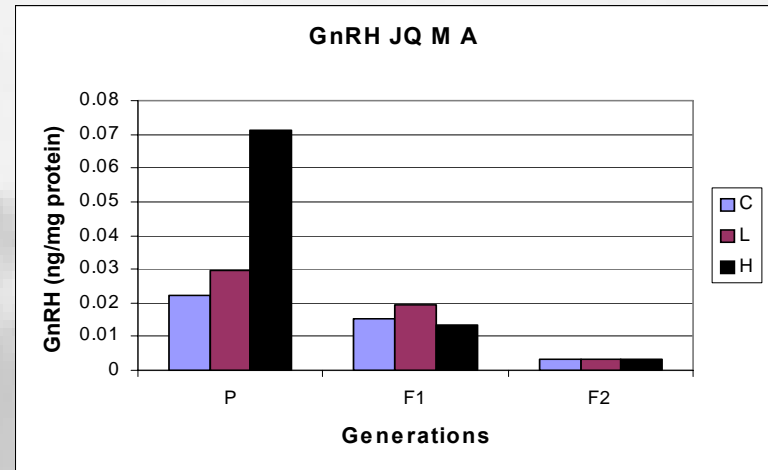
■ males

■ females

Effects of Vinclozolin and Methoxychlor on GnRH-I



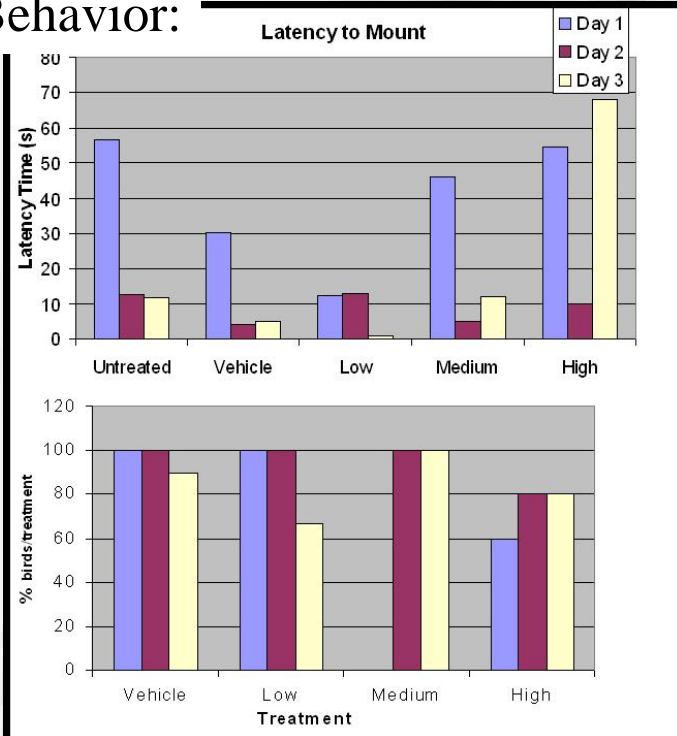
Hypothalamic GnRH-I in hatchling male Japanese quail treated with vinclozolin. Mean +SE. Capital letters denote POA, lowercase denote ME mean comparisons across treatments (McGary et al., 2001).



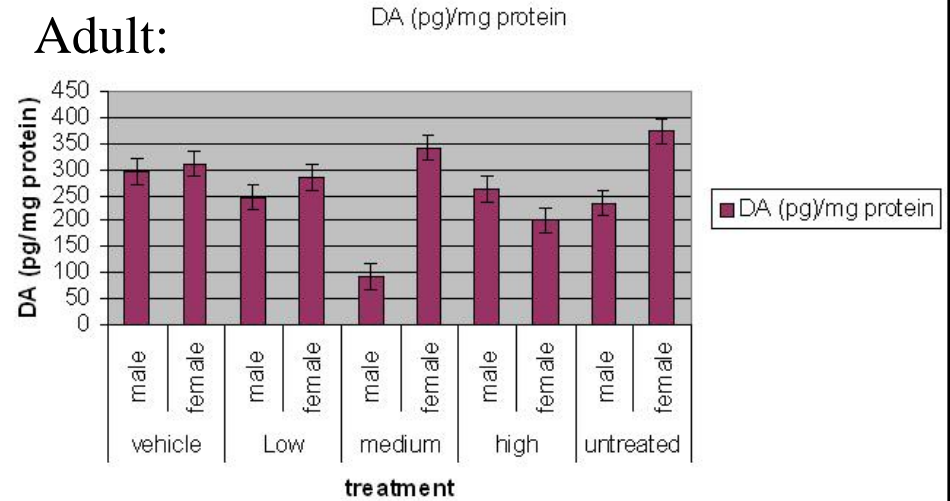
Hypothalamic POA-SL) content in hatchlings exposed to MXC during embryonic development

Effects of Embryonic Atrazine

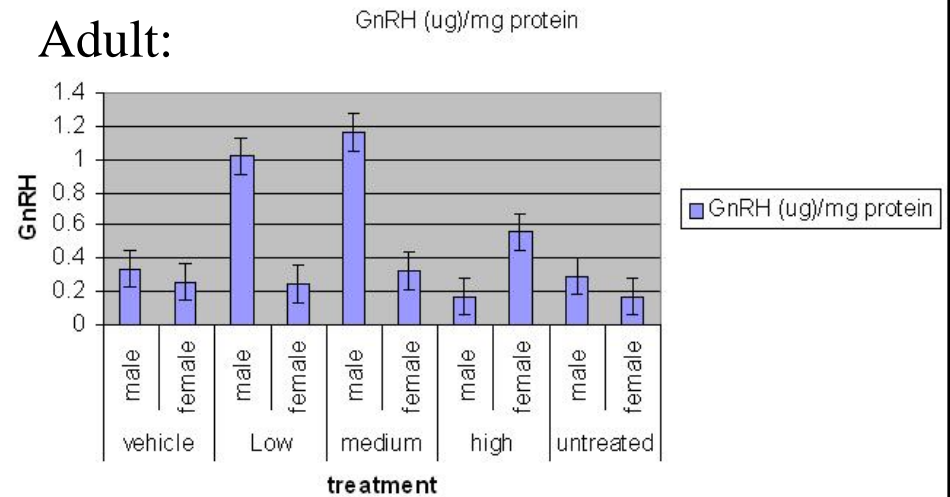
Behavior:



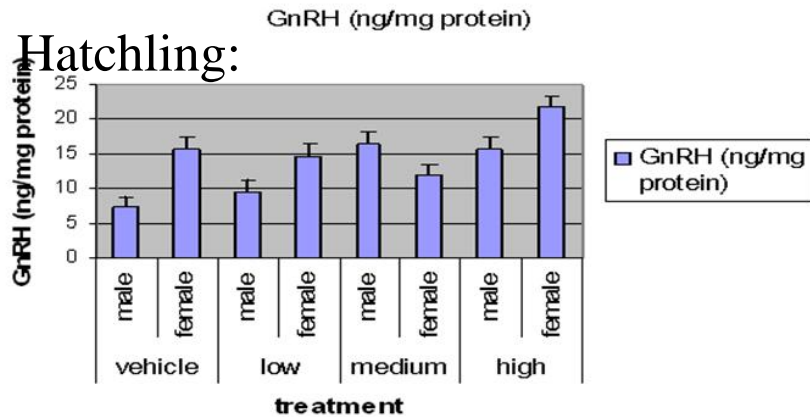
Adult:




Adult:



Hatchling:



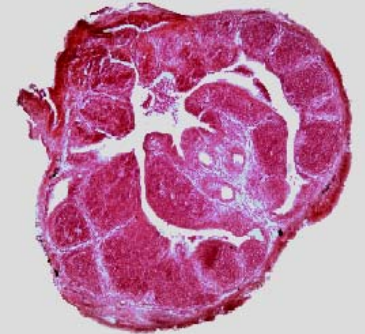
A faded, grayscale background image of two quail birds. One quail is on the left, facing right, and the other is on the right, facing left. They are both looking towards the center of the frame.

Immune system
Thyroid Axis
EDC specific indicator

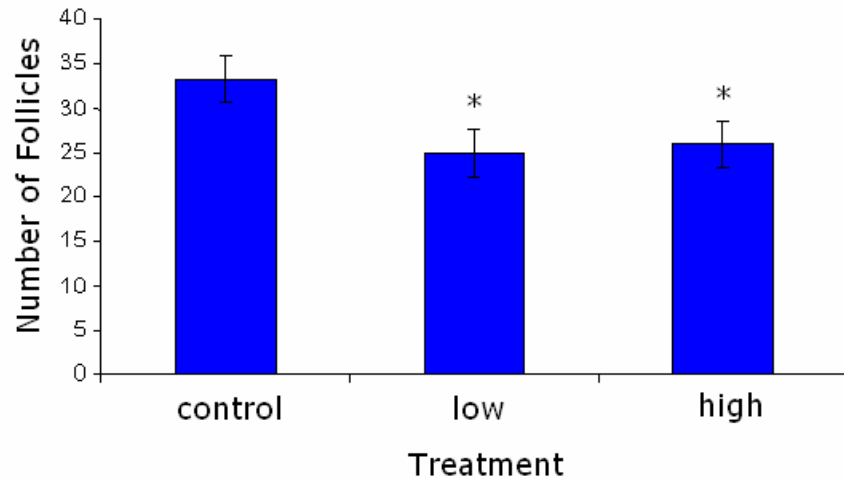
DDE Treatment Effects

(p,p'-dichlorodiphenyldichloroethane)
egg injection (E1 into yolk)

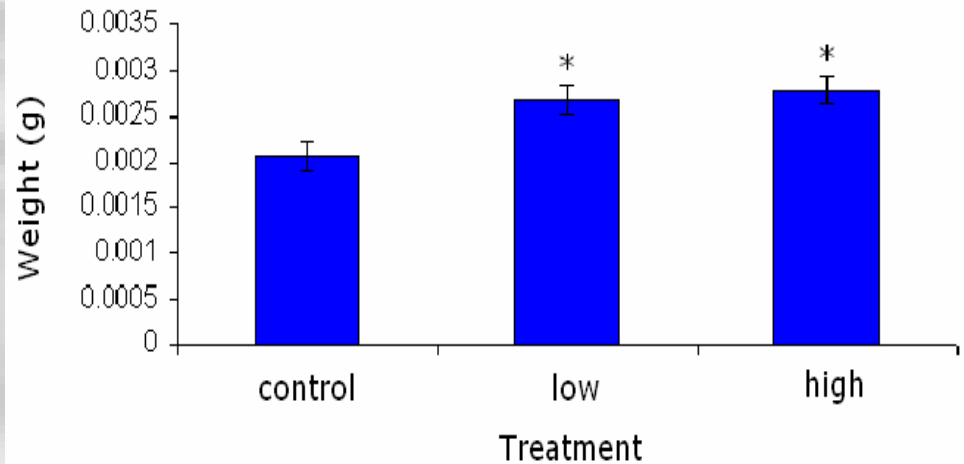
- sesame oil (vehicle control)
- 20 μg DDE (low)
- 40 μg DDE (high)



Follicle Numbers



Bursa Weights

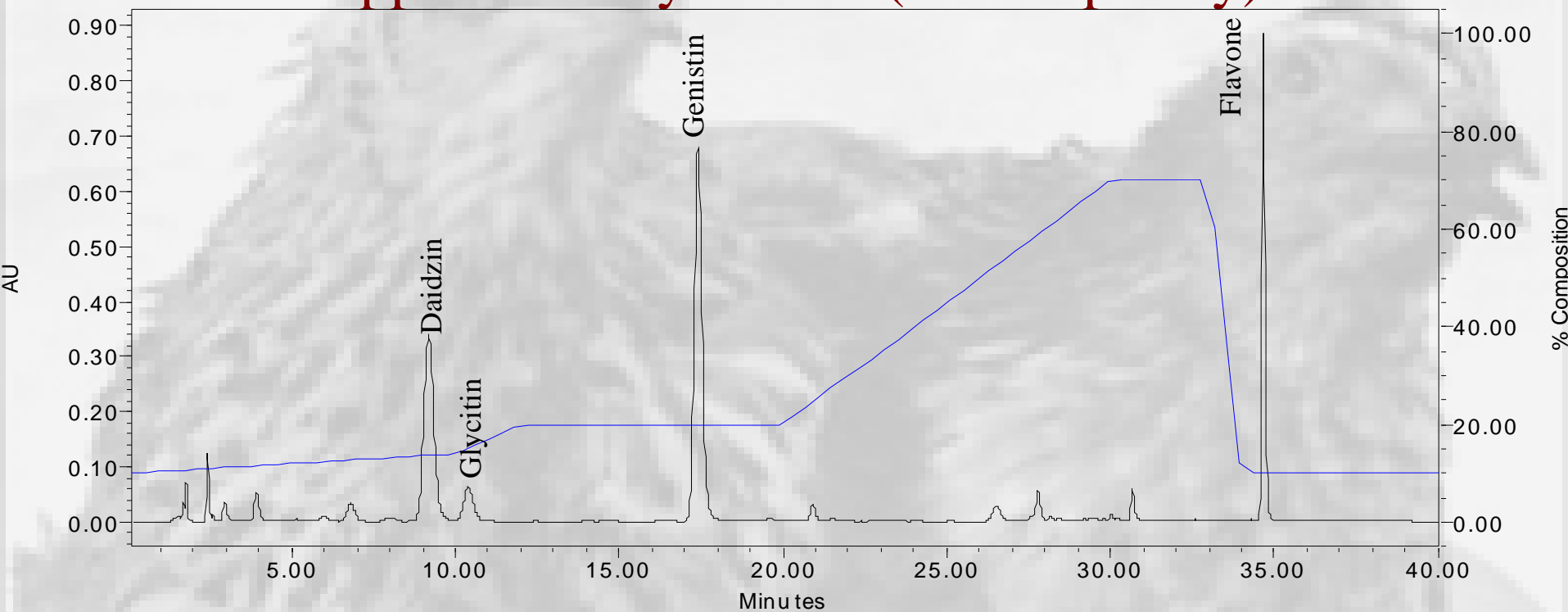


Quinn et al., 2006



Finally—soy isoflavones
Developmental effects

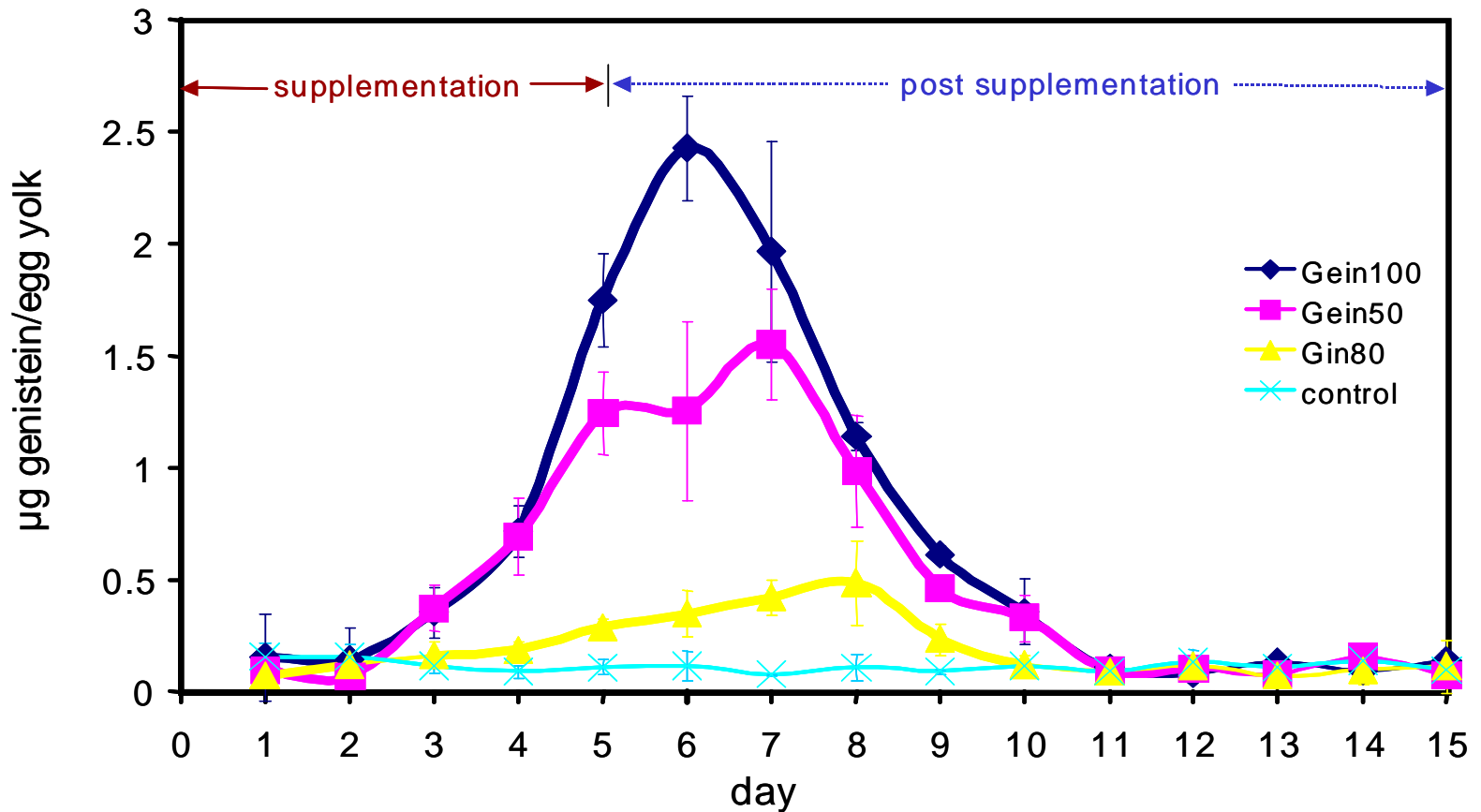
Healthy Woman—commercially available isoflavone supplement soy extract (from OptiSoy)



0.01 g sample + 25 ul flavone solution (internal standard) → a 10 ml extract solution → HPLC Injection volume: 50 μ l

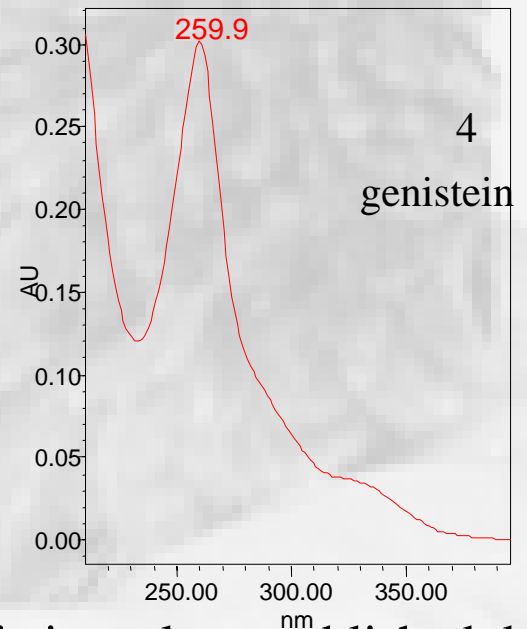
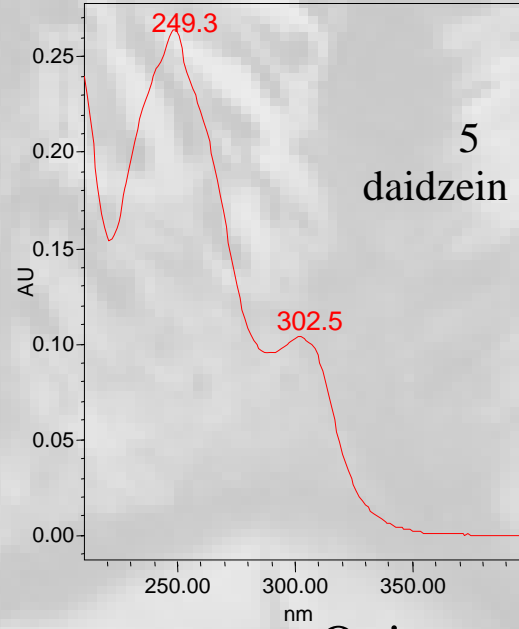
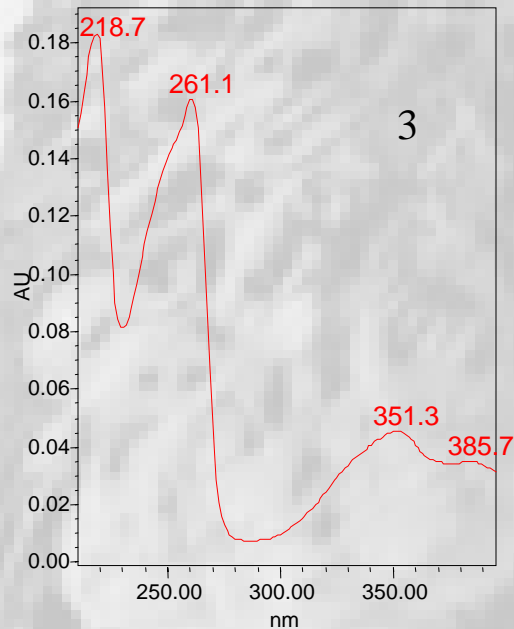
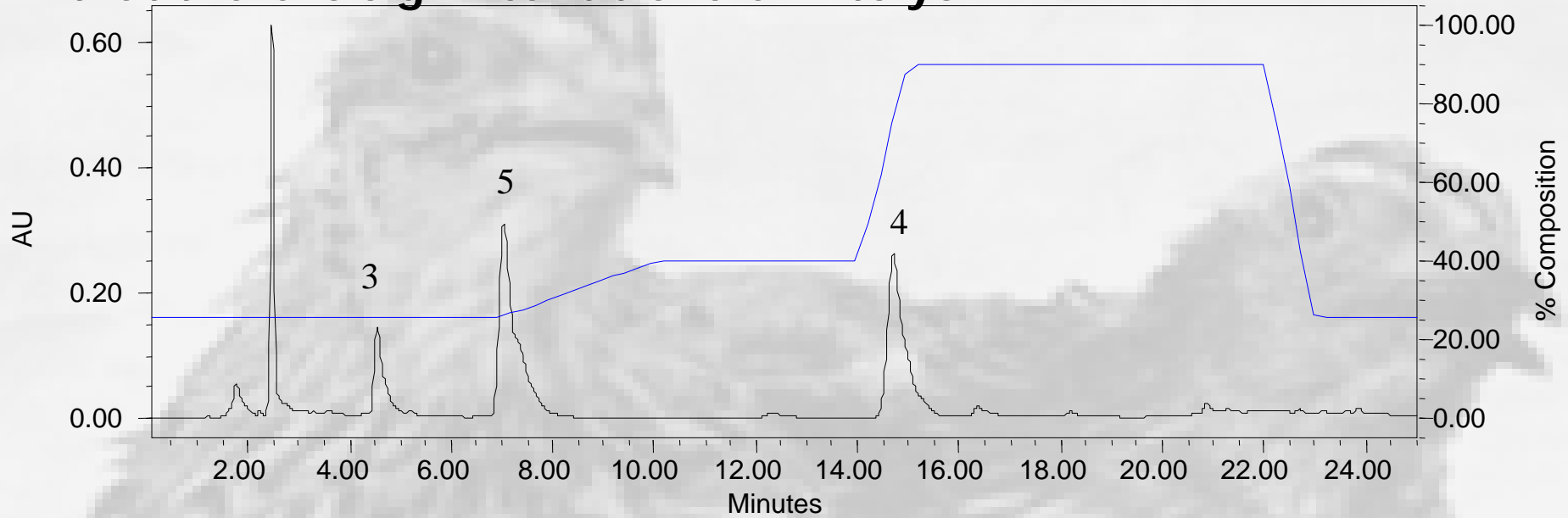
Isoflavones	Daidzin	Glycitin	Genistin
Concentration (mg/g)	35.35	3.62	42.51

Deposition of Soy Isoflavones over the 5 Day Dosing Period and Subsequent 10 Day Collection



Lin et al., 2004)

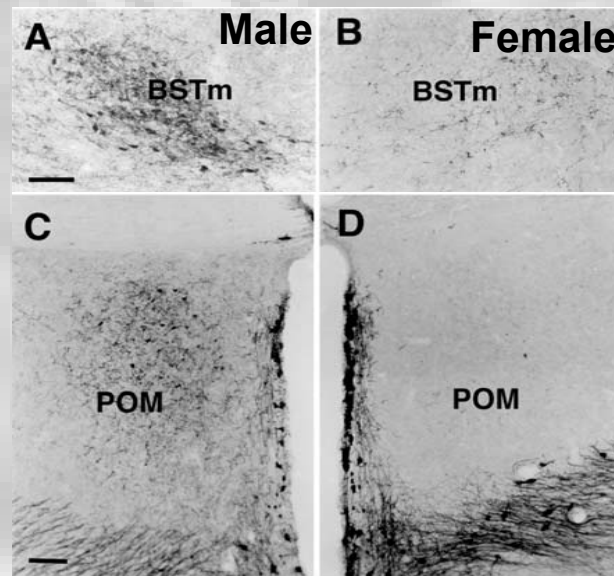
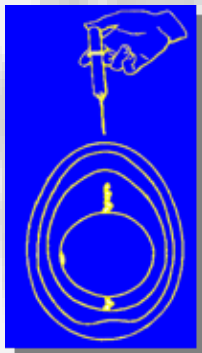
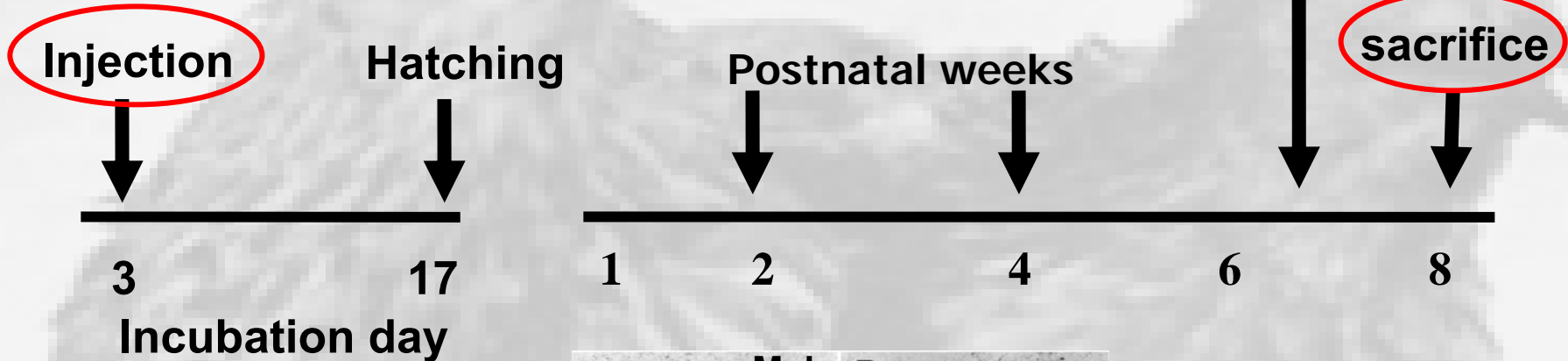
Isoflavone extract of yolk (w/ enzyme treatment) demonstrates that there is significant transfer into yolk



EXPERIMENTAL PROCEDURE

Diethylstilbestrol (DES)

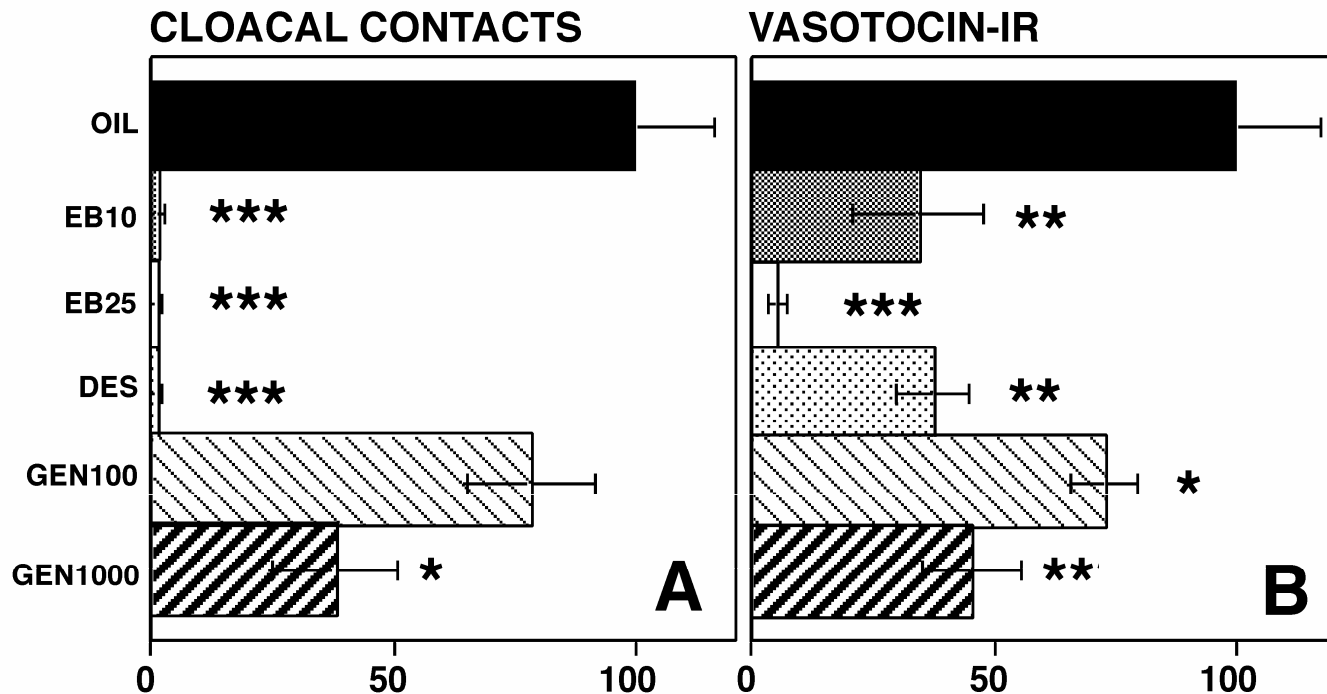
Genistein



Vasotocin (VT) provides a marker of altered sexually dimorphic systems; altered VT relates to impaired behavior and reproductive function (Panzica, Ottinger, Quinn, et al., 2007)

Genistein and DES

- DES—behavioral and neural effects
- GEN—behavioral and neural effects



(Panzica, Viglietti-Panzica, Ottinger et al., 2007)

Potential Measurement Endpoints for Avian Species:

Reliability and Sensitivity

Type of endpoint

Duration

Sensitivity

Fitness

(fertility, hatch, growth,
sexual maturation)

short and long term

less sensitive

Behavioral

(motor test, sexual behavior)

long term

very sensitive

Endocrine

(plasma/fecal hormones,
gonad morphology)

short and long term

variable

Neuroendocrine

(NE, DA, AROM, GnRH-I)

short and long term

very sensitive

from OECD Expert Group on Assessment
of EDC Effects in Birds Report, 2001.

Useful for reproductive impact, what about other systems?

Evaluating the Utility of Measurement End Points

measure/reliability	potential impact	field friendly?	agreement with literature?
lethality	limited adult population	possibly	yes/species sensitivity
deformities	reduced fitness?	yes	yes/species sensitivity
organ weights, morphology, function	altered function	yes	yes-organ specific
Hypothalamic impact	reduced fitness	yes	yes
hormones	reduced function	variable	yes—survivor effect?
maturation	late breeding/fitness	yes?	yes
immune function	fitness/survival	yes	yes
Behavior	fitness/survival	variable	yes—survivor effect?
gene expression varies with gene	high-verifying effects/new measures	potentially	??

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