

Effects of Endocrine Disrupting Chemicals in Mallard Ducks

(2008-09 SEARCA Professorial Chair Lecture)

Dr. Renato S.A. Vega
Associate Professor
Animal Breeding & Physiology Division
Animal & Dairy Sciences, UPLB

OUTLINE

- I. Milestones
- II. Mechanisms of Action
- III. State of Duck-Egg Production
- IV. Comparison of Rice-Duck Ranged and Completely Confined Management Systems
- V. Age-related Changes in Blood Vitellogenin
- VI. Conclusions/Recommendations

Definitions

- λ "An **endocrine disruptor** is an **exogenous substance** that **causes adverse health effects** in an intact organism, or its progeny, consequent to **changes in endocrine function**." (Weybridge Conference; 2003 IUPAC)
- λ "An **endocrine disruptor** is an **exogenous chemical substance** or mixture that **alters the functions(s) of the endocrine system** and thereby causes **adverse effects** to an organism, its progeny, or (sub) population." (USEPA, 1997; 2003 IUPAC)

MILESTONES

1. PD#135 Creation of Fertilizer Industry Authority in 1973.
2. PD#1144 Creation of Fertilizer and Pesticide Authority (FPA) in 1977.
3. National Poison Control and Information Service (NPCIS) in 1975.

4. Pesticide Action Network
Asia & the Pacific (PAN
AP)

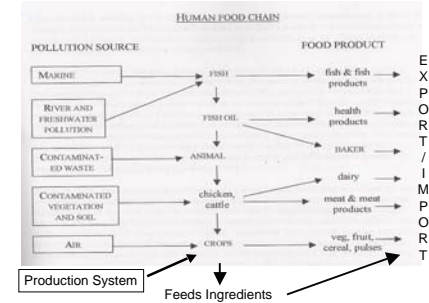
5. POPs in the environment
(IPEP, 2006)

6. Republic Act 6969 – 1990

Section 4. Objectives. - The Objectives of this Act are:

- a) To keep an **inventory** of chemicals that are presently being imported, manufactured, or used; indicating, among others, their existing and possible uses, test data, names of firms manufacturing or using them, and such other information as may be considered relevant to the protection of health and the environment;
- b) To **monitor** and **regulate the importation**, manufacture, processing, handling, storage, transportation, sale, distribution, use and disposal of chemical substances and mixtures that present unreasonable risk or injury to health or to the environment in accordance with national policies and international commitments;

Fig.1 Framework of human food chain showing contamination of fish, fish oils, meat, eggs, dairy products and crop-based products.



Modified from Alisopp et al, 2000

c) To **inform and educate** the populace regarding the hazards and risks attendant to the manufacture, handling, storage, transportation, processing, distribution, use and disposal of toxic chemicals and other substances and mixtures; and

d) To **prevent the entry, even in transit**, as well as the keeping or storage and disposal of hazardous and nuclear wastes into the country for whatever purpose.

Known EDCs

Initially there are twelve POPs identified for action under the Stockholm Convention on POPs. **Pesticide POPs** are, (1) Aldrin, (2) Dieldrin, (3) Endrin, (4) Chlordane, (5) Heptachlor, (6) DDT, (7) Taxophene and (8) Mirex. **Industrial POPs** are, (9) Hexachlorobenzene (HCB), (10) Polychlorinated Biphenyls (PCBs), (11) Polychlorinated dibenzo-p-dioxins (PCDD or Dioxins), and (12) Polychlorinated dibenzofurans (PCDF or Furans).

Prospects

1. Risk Assessment

Human health effects (breast, testicular and prostate cancer, endometriosis, abnormal sexual devt, reduced male fertility, alteration in thyroid and pituitary function, immune suppression and neurobehavior effects).

Wildlife and aquatic organisms effects are associated abnormal thyroid function and devt., decreased fertility, decreased hatching success, decreased survival of offspring, feminization and masculinization and alteration in immune and behavioral function.

(USEPA, 1997)

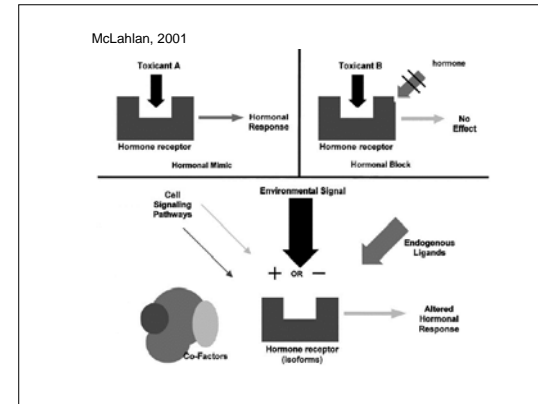
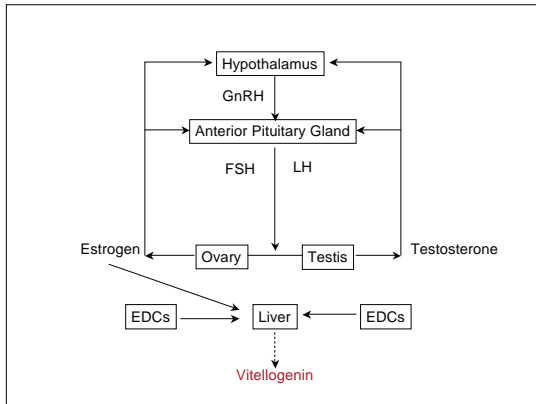
2. Risk Management

Generally focused on sources of suspected EDC's impact on environment, and to minimize the exposure to human and wildlife.

(USEPA, 1997)

MECHANISMS OF ACTIONS

1. Possible Entry
 - Oral, Dermal and Respiratory
2. Hypothalamic-Pituitary-Gonadal-Liver System Model
3. Mode of Action (McLahlan, 2001)



STATE OF DUCK-EGG PRODUCTION

1. Increase in duck population elsewhere except in Laguna.
2. The duck in Laguna experience dual peak.
3. Duck egg production of 65% is critical if given commercial feeds.
4. Development of expensive feeds does not match the breed nutritional requirements.
5. Country's total duck meat and egg production declined by 7.68 and 6.08%, respectively.

Conventional feeds from duck farms in Victoria, Laguna

- Conventional feeds composed of tiny gastropods and the edible freshwater clam



Duck Breeds



RICE-DUCK RANGED VS COMPLETELY CONFINED PRODUCTION SYSTEMS

Exploratory Research (ANSC 285)

- λ Comparison of rice-duck ranged and confined ducks production systems (n=6)
- λ Collect duck samples (5 males and 5 females)
- λ Collect feed samples (commercial feeds and other feed ingredients)
- λ Hepatosomatic Index (HIS)
- λ Gonadosomatic Index (GSI)
- λ Document abnormalities in liver and reproductive organs

Pesticide & Heavy Metal Analysis

- λ Sampling at various locations
- λ Duck and feeds samples (commercial and conventional).
- λ Three commercial feeds in two forms, i.e. pellet and mash.
- λ Water sample at Laguna Lake

Laguna Lake and the surrounding provinces and municipalities



Results

Hepatosomatic Index (Ranging vs Confined)

- Heavier for Ranging (2.64 vs 2.41%)
- Layer ducks (3.34 vs 2.93%)
- Drakes (1.94 vs 0.88%)

Gonadosomatic Index (Ranging vs Confined)

- Comparable (4.4 vs 4.43%)
- Drakes better when ranged (2.89 vs 1.41%)
(less stress and availability of Vit. D)

Abnormal Liver

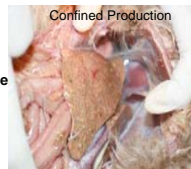
1. Liver Cirrhosis



2. Hematoma



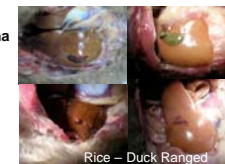
3. Tumor-like structure



1. Liver Cirrhosis

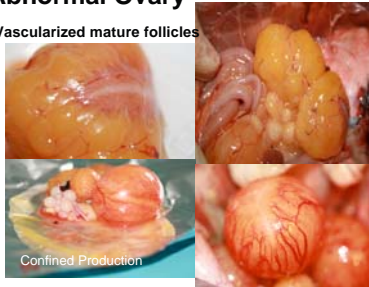


2. Hematoma



Abnormal Ovary

1. Vascularized mature follicles



2. Vascularized and dispersed blood formation



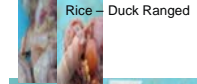
3. Necrotic follicles (dark colored ova)



4. Dispersed yellow pigment portion of the follicles



1. Empty oocyte



2. Mature follicle about to be laid and few growing follicles



3. Close proximity of mature ova at the isthmus



4. Yellow pigment dispersed between immature follicles



Pesticide Residues		HG - Pellet	HG - Mash	PBL - Pellet	PBG - Mash	AL - Pellet	EMDL*
Lindane	β-BHC	→ 150	88	125	<EMDL	130	0.5
	γ-BHC	→ <EMDL	80	<EMDL	<EMDL	90	0.7
	δ-BHC	→ 55	41	20	<EMDL	50	0.4
Chlordane	γ-Chlordane	→ 1.4	28	<EMDL	0.65	1.2	0.6
	α-Chlordane	→ <EMDL	19	<EMDL	<EMDL	0.9	0.8
DDT	p,p' - DDD	→ <EMDL	<EMDL	<EMDL	<EMDL	3.7	0.3
	o,p' - DDT	→ <EMDL	<EMDL	<EMDL	<EMDL	4.0	0.5
	p,p' - DDT	→ <EMDL	<EMDL	<EMDL	7	<EMDL	0.9
Nonachlor	trans-Nonachlor	→ <EMDL	16	<EMDL	<EMDL	<EMDL	0.7
	cis-Nonachlor	→ <EMDL	2.6	<EMDL	<EMDL	<EMDL	0.9
Heptachlor	→ <EMDL	8.8	<EMDL	<EMDL	<EMDL	<EMDL	0.5
Methoxychlor	→ 1.23	<EMDL	50	3.52	<EMDL	<EMDL	0.9
Aldrin	→ <EMDL	<EMDL	<EMDL	<EMDL	<EMDL	<EMDL	0.5
Hexachlorobenzene	→ <EMDL	<EMDL	0.34	<EMDL	<EMDL	<EMDL	0.3
Endrin	→ <EMDL	<EMDL	<EMDL	3.7	<EMDL	<EMDL	2
Dieldrin	→ <EMDL	<EMDL	<EMDL	<EMDL	<EMDL	<EMDL	2

AGE-RELATED CHANGES IN VITELLOGENIN

Molecular techniques

- λ VTG mRNA
- λ VTG peptide detection using SDS-PAGE(Western Blotting)

Figure 5. Agarose gel electrophoresis of reamplified PCR products from liver samples of Philippine White Mallard and Pekin (*Anas platyrhynchos*), utilizing chicken (CV4421F) primer.

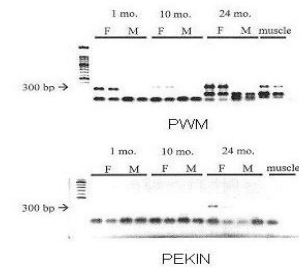


Figure 6. Putative vitellogenin (VTG) peptide band at 240 kDa using Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS – PAGE) from the liver of male and female Mallard ducks at ages 5, 10 and 16 months old.

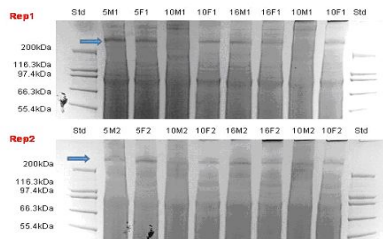


Figure 7. Putative vitellogenin (VTG) peptide band at 240 kDa using Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS – PAGE) from the plasma (100x buffer dilution) of male and female Mallard ducks at ages 5, 10 and 16 months old.

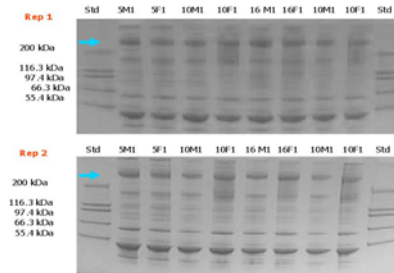
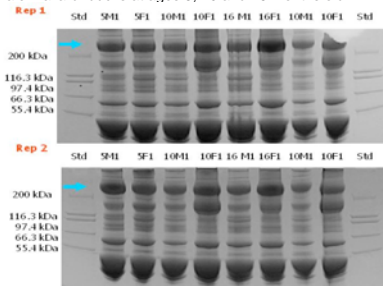


Figure 8. Putative vitellogenin (VTG) peptide band at 240 kDa using Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS – PAGE) from the plasma (10x buffer dilution) of male and female Mallard ducks at ages 5, 10 and 16 months old.



CONCLUSIONS

1. Historical Background (lessons learned)
Government policies are almost always **reactionary**, foreign **favored** and **dependent**
Case 1 – Fertilizer Industry Authority,
Case 2 – Endosulfan
Case 3 – Lawsuit against Dr. Romeo Quijano

2. State of Duck Industry

- Y Declining duck egg and meat production by 6.06 and 7.68%, respectively
- Y Presence of several liver and reproductive abnormalities
- Y Commercial feed cost is breakeven with percent egg production at 65%.
- Y Presence of pesticide residue in commercial feeds

3. Abnormalities and Implications to Human Health

- Y Lindane residues (41-150 ppb) in commercial feeds
- Y Lindane is a health hazard
- Y Heavy metal cadmium in freshwater clam registered at 500 ppb.
- Y Chlordane, DDD, DDT, Endrin, Heptachlor and Mirex are present in small amounts.

4. Chicken vitellogenin primer can be used for duck vitellogenin mRNA determination.

5. Male vitellogenin detection can be used as a biomarker for exposure of oviparous animals to EDCs.

ACKNOWLEDGMENT

- λ Students of ANSC 285 (2006)
- λ Office of Vice-Chancellor for Res. & Extension (Basic Res.)
- λ UP Systems Emerging S & T Grant (Vice-Pres. Amelia Guevara)
- λ Dep't. of Agric. – Bureau of Agric. Res. (Dir. Nicomedes Eleazar)
- λ Undergraduate Students
Ms. Lovely Lawas – Mol. Biol. & Biotech
Mr. Mark Purugganan – Animal Science

THANK YOU VERY MUCH!

“It is better to light a candle
than to curse the darkness”

Eleanor Roosevelt
1884 – 1962