KASETSART UNIVERSITY

“Heavy Metals: a possible health risk through free grazing duck farm in Thailand”

Assoc. Prof. Phitsanu Tulayakul,

DVM, MSc., Ph.D., DTBVPH

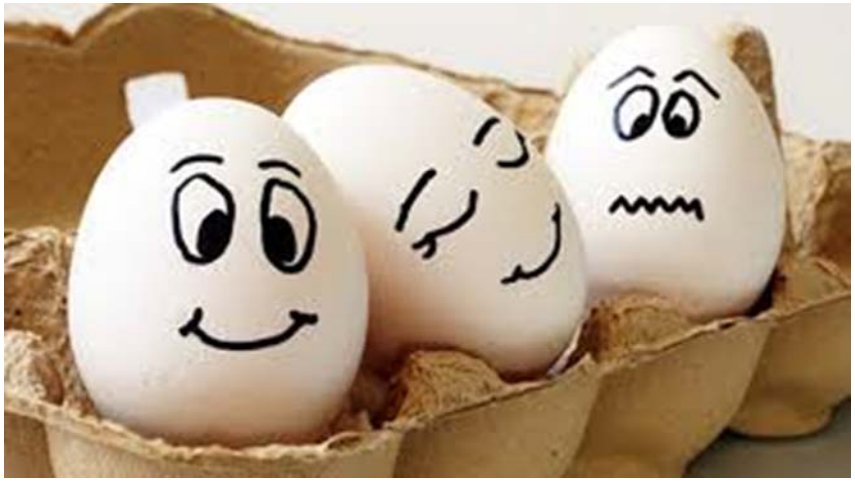
Introduction

- Heavy metals are normally present in the environment, anthropogenic activities may increase metal concentrations in aquatic environments and causes of hazard to ecological systems, human and animal health [Stine & Brown, 2006; Sharma et al., 2014; Abdulla et al., 2015; Kim et al., 2016].

- Food??

- Metals such as lead (Pb) and cadmium (Cd) are toxic at lower concentrations and can be consumed through dietary supplements.
- Cd induces kidney dysfunction, osteomalacia and reproductive deficiencies.
- Pb is known to induce renal tumors and neurological and hematological effects, reduce cognitive development, and increase blood pressure and cardiovascular disease in adults.

- Cobalt (Co) causes erythropoiesis and chronic oral administration of high levels of Co can cause goiters.
- Chromium (Cr^{+6}) is a human carcinogen and produces a variety of toxic effects and it can be deposited on land and water and eventually on sediments. It causes damage to cellular components, generation of free radicals, and the formation of DNA adducts
- However, some trance element of Fe, Cu and Mn are essential component in human and animal body.



- The number of duck in Thailand was about 13.5 million animals in 2015 (50% was egg duck)
- Thailand is 1 of the 5th leader of duck meat exporter of the world.
- Pekking, Cherry Valley breed are popular meat ducks while Khaki Campbell and native Nakhon Pathom breed are popular egg ducks.

Purpose of study

- Since there is limited information of heavy metal and risk of aflatoxin concerning the possible contamination of egg products from free grazing duck farms.
- Thus, the aim of the present study was to evaluate the levels of aflatoxin and heavy metal contamination in the duck eggs and liver tissue, water, and feed from free grazing duck farms in the central region of Thailand.

Research Methodology I

- Survey of raising condition and transportation route of free grazing duck of each farmer (December 2010-August 2011)
- Collect blood and offal samples from 3 slaughter houses of which samples come from various provinces of Thailand (December 2010-February 2011)

Research Methodology II

- **10 each of** Liver tissue, offal, eggs and whole blood were randomly taken, kept in 4˚C before analyzing for lead and cadmium by Atomic Absorption spectrophotometer (AA).
- Pooled sample of 10 sampling of brand rice, soils and water in paddle field (Before/After) release in of free grazing duck were taken and analyze for heavy metal using

Determination of Lead and Cadmium in Duck's egg

- According to method 3050B (http://www.epa.gov/sw-846/3_series.htm) with modifications.

Determination of Lead and Cadmium in Duck's Liver

- According to Handbook: Analytical Method for Graphite Atomizers, Hitachi with modifications.

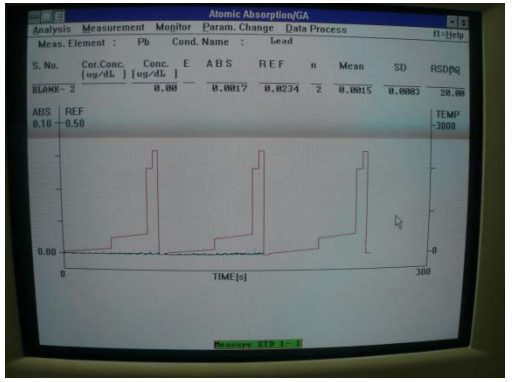
Research Methodology III

AFB1 determination in Soils and brand rice

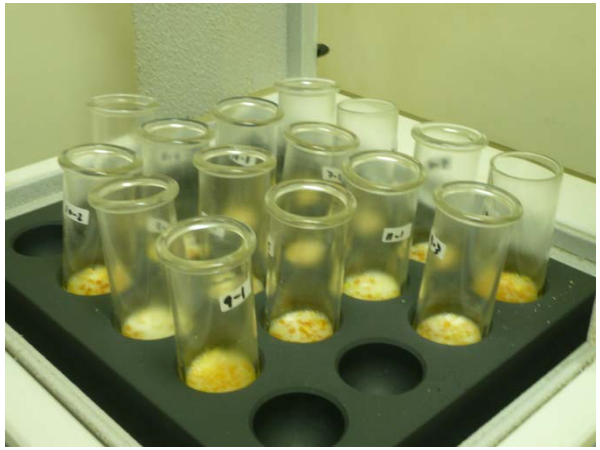
Using Veratox HS NEOGEN® (Quantitative Aflatoxin High Sensitivity Test)

AFB1 determination in liver tissue and duck eggs

Extraction by method of Gathumbe et al., 2003 and precipitated of Methanol-Acetone-PBS layer ⟶ Analyzed by Veratox HS NEOGEN®

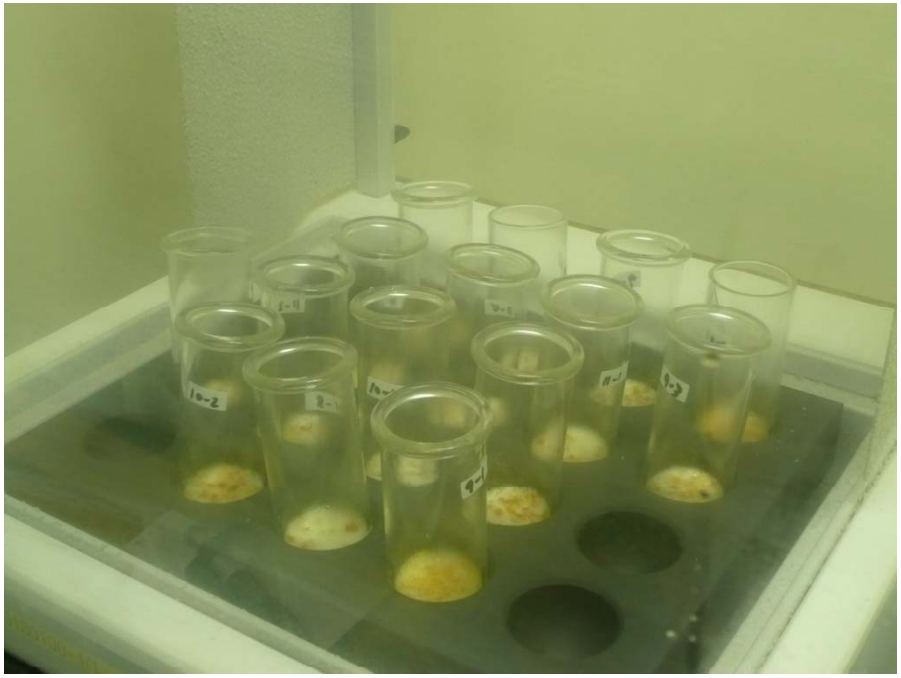


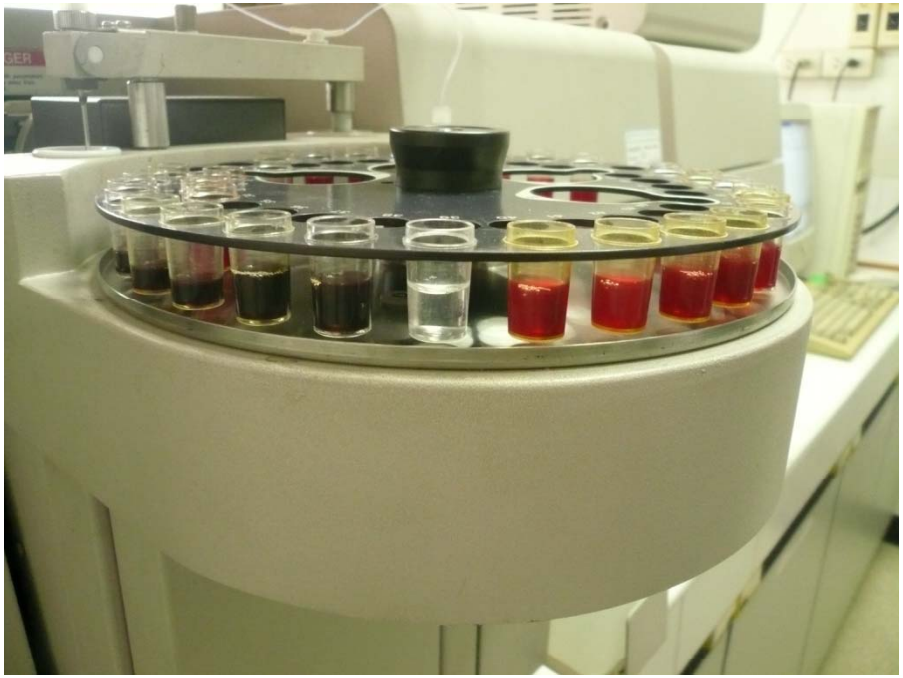
Veratox HS NEOGEN® : LOD of 0.5 ppb



LOQ of 1.0 ppb

Range of Quantification (1.0-8.0 ppb)



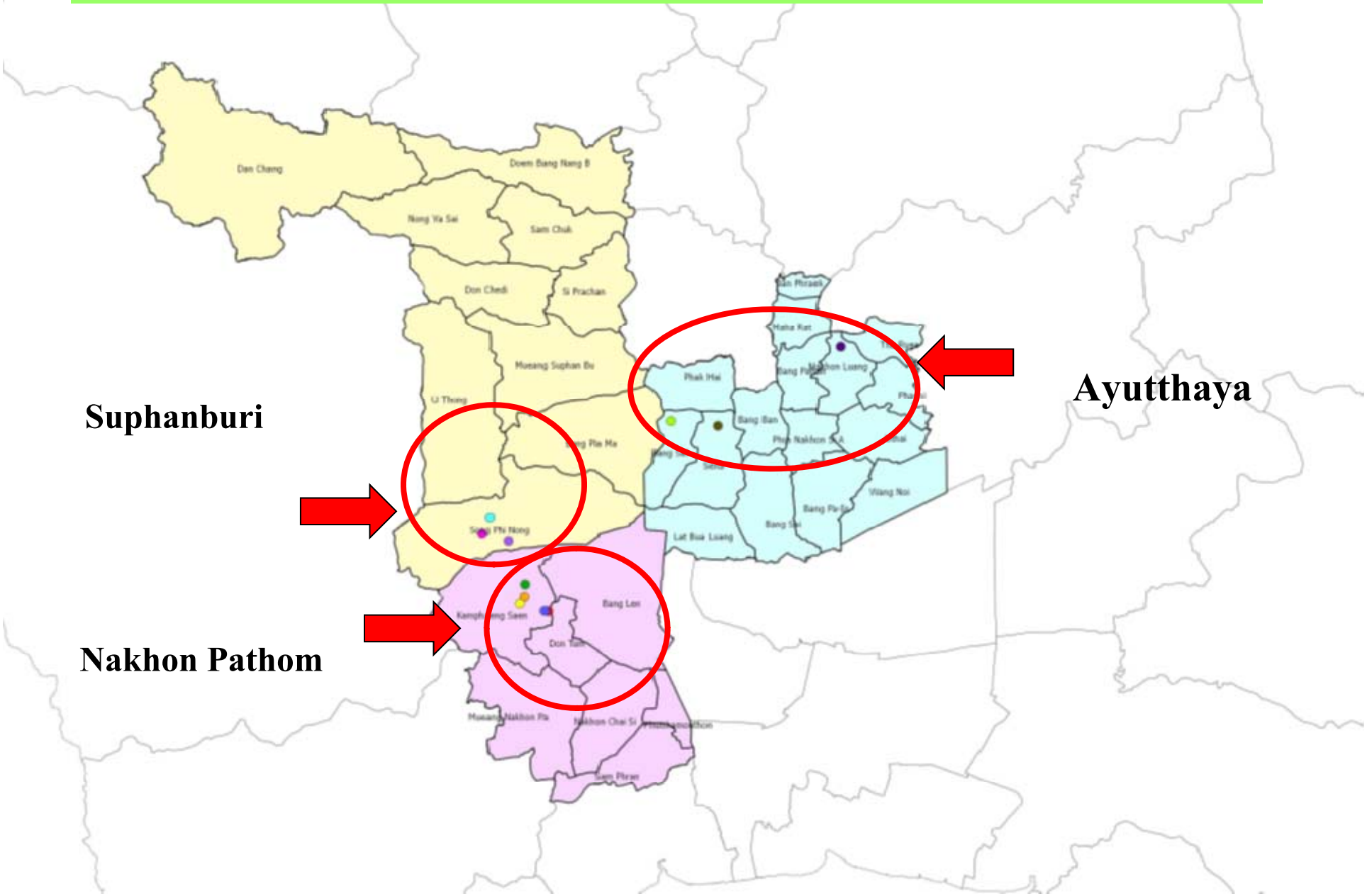



Atomic Absorption/GA
Analysis Measurement Monitor Param. Change Data Process
f1=Help
Meas. Element : Pb Cond. Name : Lead
S. No. Cor.Conc. [ug/dL] Conc. [ug/dL] E ABS REF n Mean SD RSD[%]
BLANK- 2 0.00 0.0017 0.0234 2 0.0015 0.0003 20.00
ABS REF
0.10 0.50
TEMP
3000
0.00
0
0
TIME(s)
300
Measure STD 1- 1

European Commission Regulation (EC) No 1881/2006

- Lead levels in meat from cows, ewe, pig and poultry (exclude offal) maximum level of ≤ 0.1 mg/kg (wet weight), its offal of ≤ 0.5 mg/kg (wet weight) and Cadmium (exclude offal) maximum level of ≤ 0.050 mg/kg (wet weight) and in liver of ≤ 0.20 mg/kg (wet weight)
- Eggs no legislation limits for EC of these Heavy metal
- No legislation limits for heavy metal in meat offal and eggs in Thailand

GIS (Geographical Information System) of 11 studies area:



Results

Table 1 The results of AFB1 analysis in the liver, intestine, and eggs from free grazing ducks using ELISA

AFB1 (ng g^{-1})	Liver	Intestine	Egg yolk	Egg white
Total number, *n* (%)	99 (100)	100 (100)	90 (100)	90 (100)
Detectable number, *n* (%)	72 (72.72)	26 (26)	58 (64.44)	0 (0)
Non-detectable number, *n* (%)	27 (27.27)	74 (74)	32 (35.55)	90 (100)
Average	1.60	1.31	1.87	0
Standard deviation	0.73	0.23	0.99	0
Maximum level	3.04	1.73	4.56	0
AFB1 (ng g^{-1})				
Limit of detection	0.5			
Limit of quantization	1			
Range of quantization	1–8			

Table 2 The levels of Pb and Cd contamination in blood samples collected from free grazing ducks from slaughterhouses and analyzed by Atomic Absorption.

Blood sample	(n)	Detectable(%)	ND(%)	Mean	SD	Maximum level	Detection limit (ppb)	Limit of Quantification (ppb)
Lead	135	132 (97.77%)	3(2.33%)	61.222	53.874	279.70	5.3	40.0
Cadmium	135	57(42.22%)	78(57.78%)	1.996	0.897	6.00	0.95	-

ND: Non-detectable

SD: Standard deviation

Table 3. The levels of Pb and Cd contamination in eggs collected from free grazing ducks.

Egg sample	(n)	Detectable(%)	ND(%)	Mean	SD	Maximum level	Limit of Quantification (ppb)
Lead	125	2(1.60%)	123(98.40%)	118.61	22.25	134.340	3.60
Cadmium	124	5(4.04%)	119(95.96%)	13.84	9.06	29.720	0.16

ND: Non-detectable

SD: Standard deviation

Table 4. The levels of Pb and Cd contamination in liver tissue collected from free grazing ducks.

Liver sample	(n)	Detectable(%)	ND(%)	Mean	SD	Maximum level	Standard limit (ppt)
Lead	94	88(93.61%)	6(6.39%)	97.74	96.106	606.16	500,000
Cadmium	100	100(100%)	0	2185.57	3827.078	22,946.20	500,000

ND: Non-detectable

SD: Standard deviation

Table 5. The levels of Pb and Cd in water from rice paddy fields before and after allowing free ranging ducks to graze and comparison of the detection levels with standard limits.

Type of water	Sample (n)	Provinces	Lead (ng/g)	Standard limit[1]	Cadmium (ng/g)	Standard limit[1]
Before grazing	1	Nakhon Pathom	4.25	Lead < 200 ng/g	11.64	Cadmium < 30 ng/g
	2	Nakhon Pathom	9.37		-	
	3	Nakhon Pathom	-		-	
	4	Nakhon Pathom	-		7.58	
	5	Suphanburi	-		-	
	6	Suphanburi	-		0.24	
	7	Suphanburi	-		0.85	
	8	Nakhon Pathom	3.84		0.26	
	9	Ayudhaya	-		-	
	10	Ayudhaya	-		0.28	
	11	Ayudhaya	7.59		0.25	
		Mean ± SD	2.28 ± 3.48*		1.92±3.92	
After grazing	1	Nakhon Pathom	44.89	Lead < 200 ng/g	0.18	Cadmium < 30 ng/g
	2	Nakhon Pathom	129.70		-	
	3	Nakhon Pathom	23.04		-	
	4	Nakhon Pathom	34.91		0.41	
	5	Suphanburi	53.71		2.57	
	6	Suphanburi	26.43		1.97	
	7	Suphanburi	43.86		0.24	
	8	Nakhon Pathom	7.51		1.01	
	9	Ayudhaya	-		-	
	10	Ayudhaya	14.43		0.85	
	11	Ayudhaya	8.25		0.77	
		Mean ± SD	37.85 ± 34.70*		0.72 ± 0.85	

- Consuming of eggs from free grazing duck having toxicity risk of **lead** contamination of 5.52 times until 15.98 times especially for children in long term.
- (Tolerable range for lead intake/10 body weight).

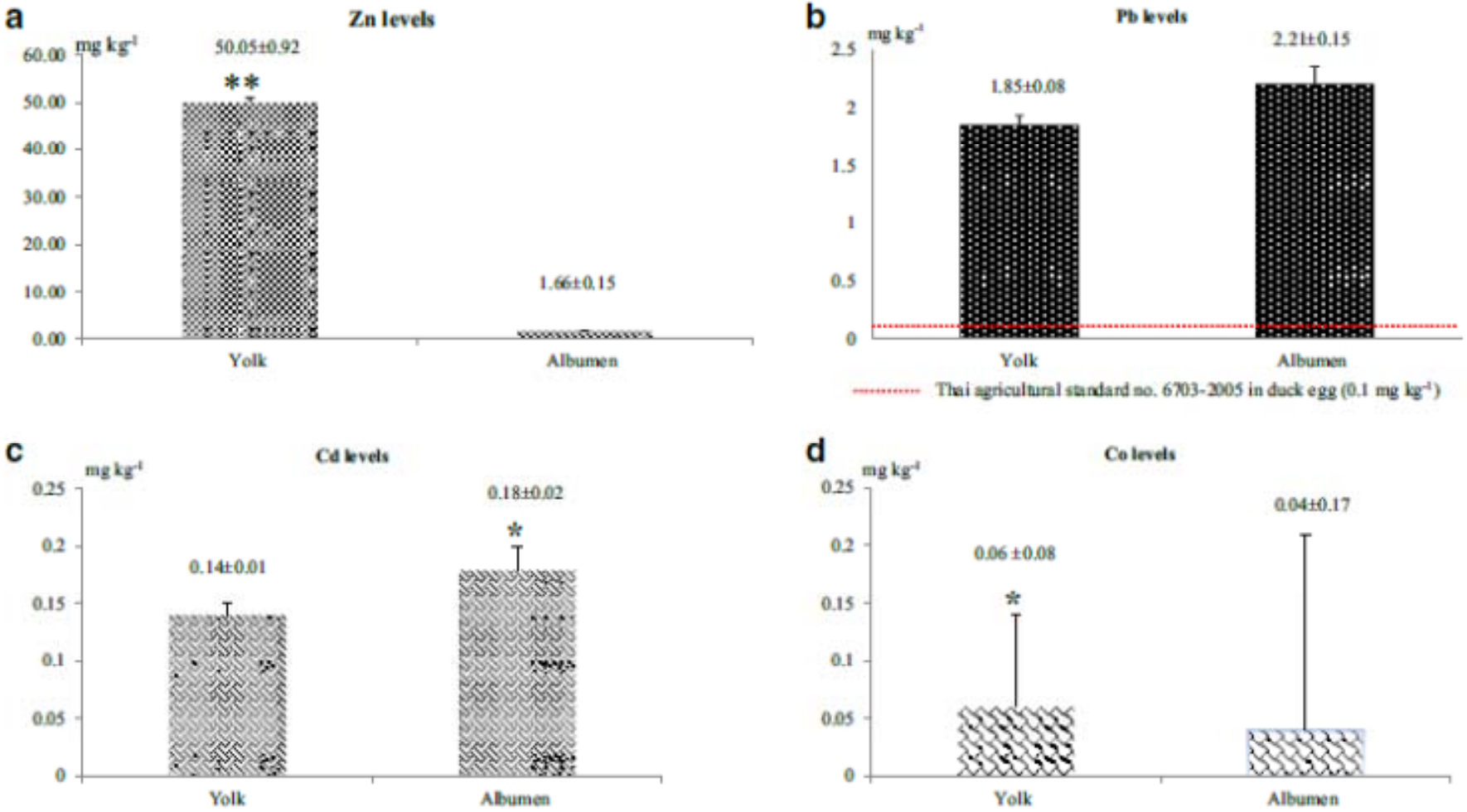
- Cadmium levels in eggs were high compared with the standard limits, by the way, it needs to do further survey.

- Anyway, the highest levels found was higher than 5 µg/day which still at risk for consumer if consuming eggs with highest cadmium found was 5.9 times /day.

Conclusion

- The highest levels of aflatoxin were detected in eggs yolk, then in liver and intestine, respectively.
- Aflatoxins were not detected in any of eggs white albumin, whereas aflatoxin contamination was remarkably found in the eggs yolk.
- Pb and Cd contamination mainly detectable in duck liver tissue, and the maximum levels of Pb and Cd in duck liver tissues were higher than in duck eggs.
- Pb contamination in the water from rice paddy fields after grazing was significantly higher than water samples taken before grazing.
- However, Pb contamination in eggs, blood and duck liver samples were still in the line which considered to be safe for consumer.

II


a
Zn levels
mg kg-1
60.00
50.00
40.00
30.00
20.00
10.00
0.00
50.05±0.92
**
1.66±0.15
Yolk
Albumen
b
Pb levels
mg kg-1
2.5
2
1.5
1
0.5
0
1.85±0.08
2.21±0.15
Yolk
Albumen
Thai agricultural standard no. 6703-2005 in duck egg (0.1 mg kg-1)
c
Cd levels
mg kg-1
0.25
0.2
0.15
0.1
0.05
0
0.14±0.01
0.18±0.02
*
Yolk
Albumen
d
Co levels
mg kg-1
0.25
0.2
0.15
0.1
0.05
0
0.06 ±0.08
*
0.04±0.17
Yolk
Albumen

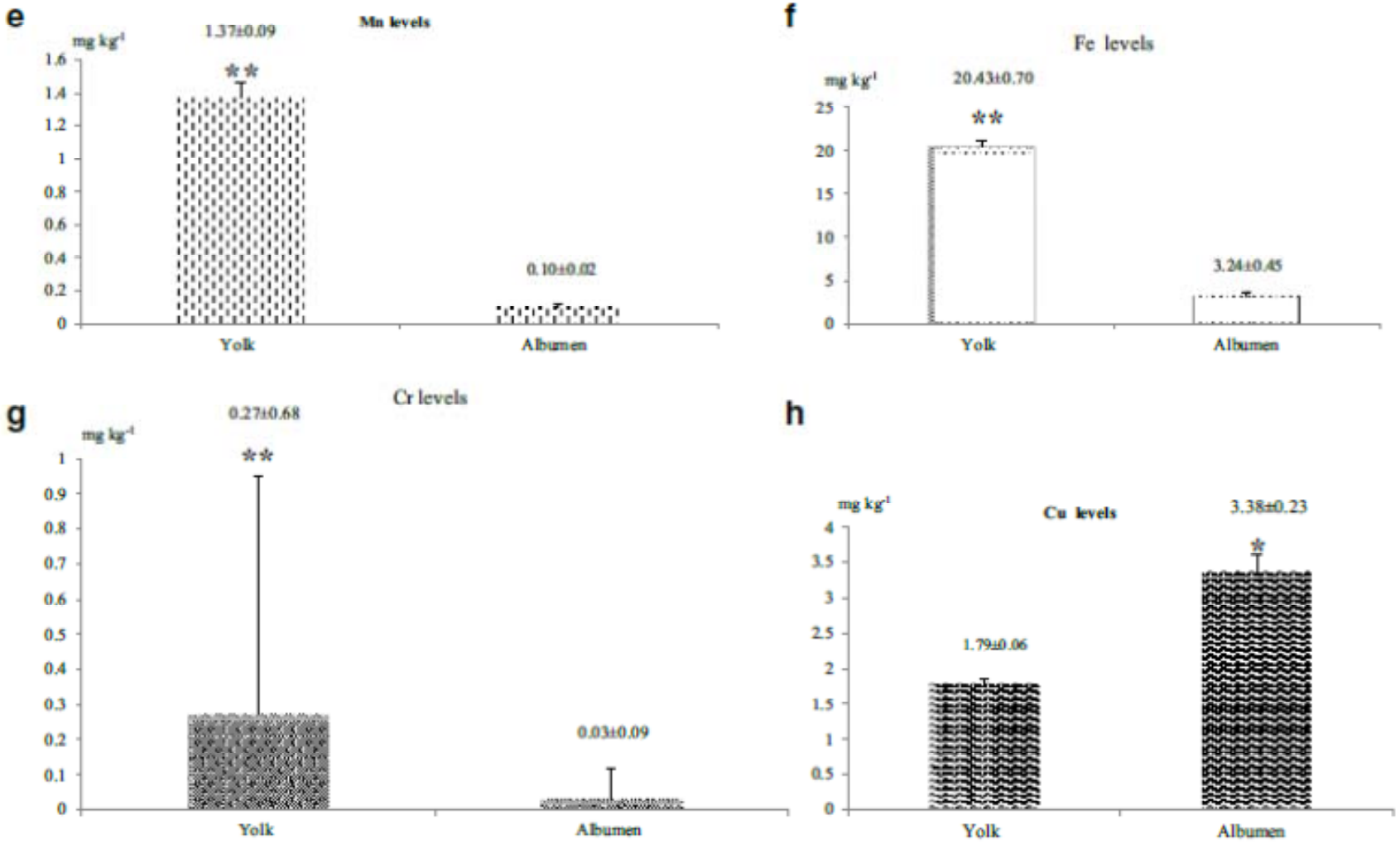


Fig. 1 The mean ± standard deviation of heavy metals determined in yolk and albumen of duck eggs. It shows that Cu and Cd in albumen higher than in yolk, but, Pb in albumen was higher than in yolk with no significant difference. * = significant difference at $P < 0.05$, ** = significant difference at $P < 0.001$

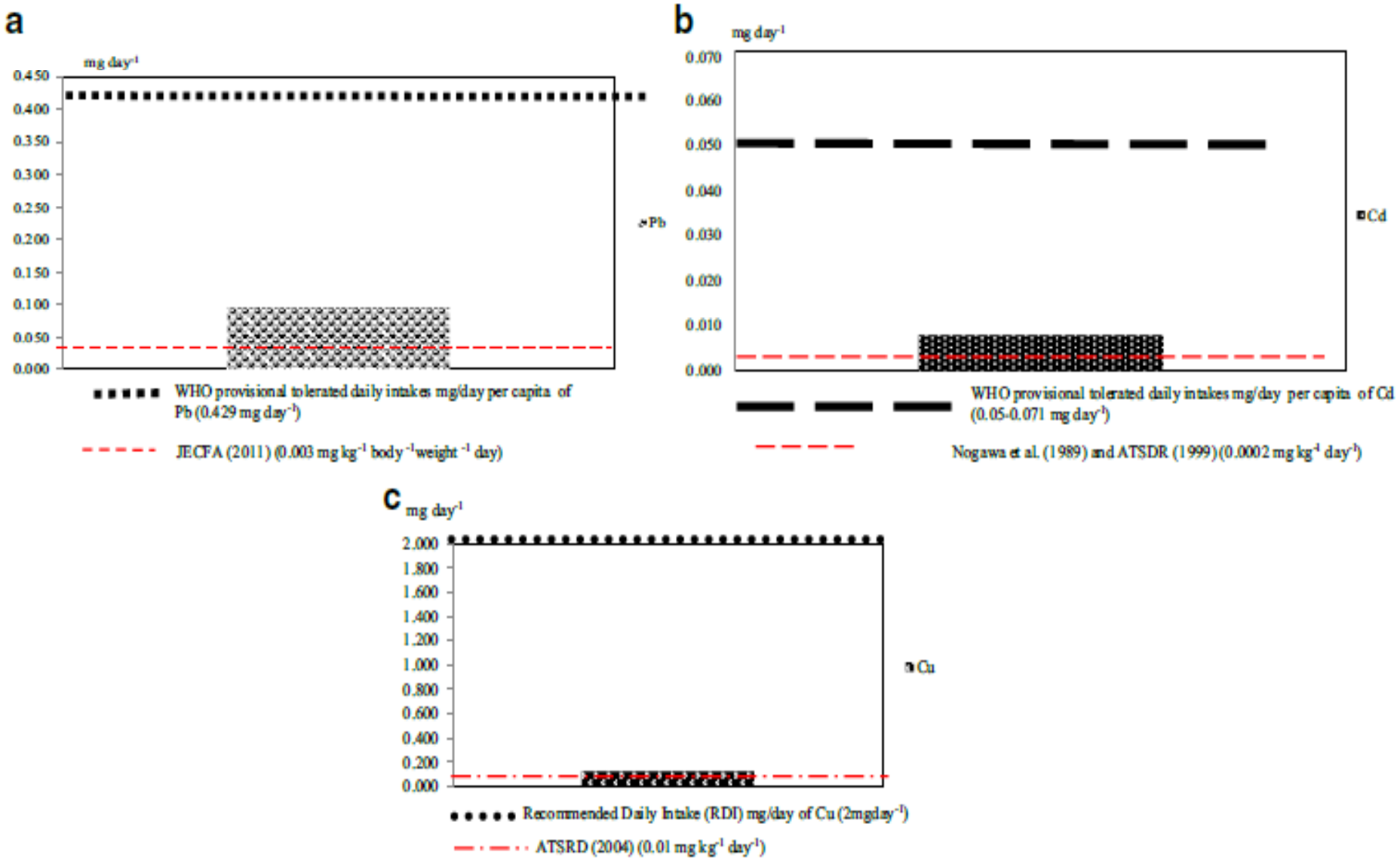


Fig. 2 The calculation of average daily intake based on duck egg consumption according to the WHO provisional tolerated daily intake and RDI. It shows an average daily intake of Pb, Cd, and Cu presented at lower levels than the standard limit

Human Health Risk Assessment

- **Estimated Daily Intake**

  $$EDI = FIR \times C/BW$$

- **Incremental lifetime cancer risk (ILCR)**

  $$ILCR = EDI \times CSF$$

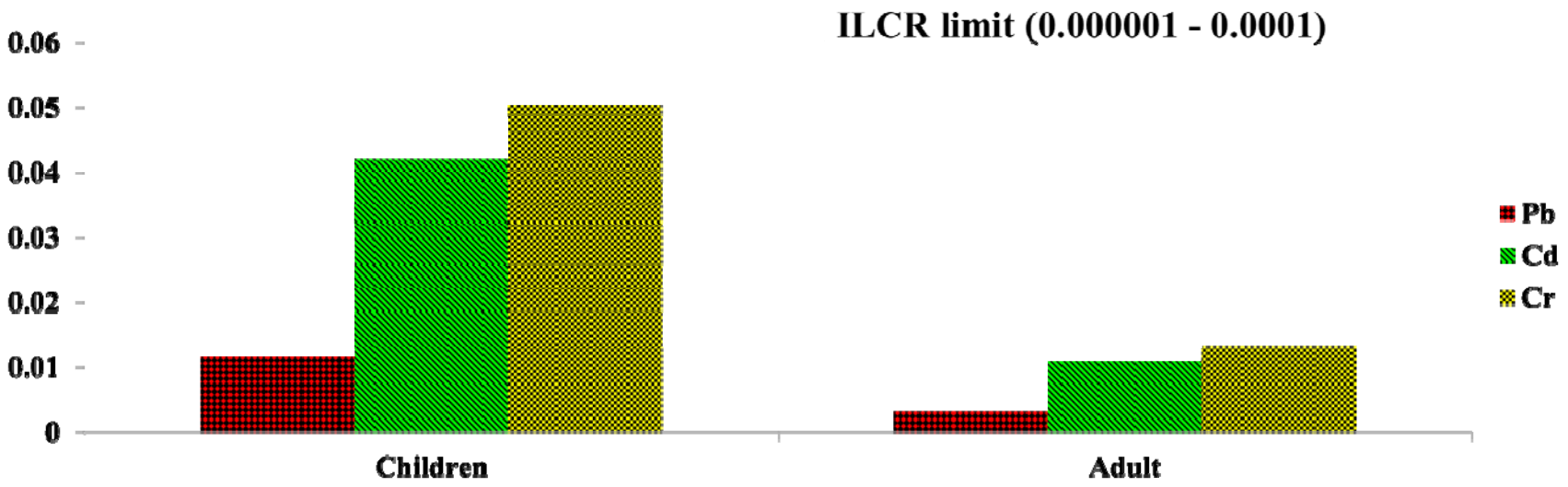
- **Target Hazard Quotient**

  $$THQ = (EFr \times ED \times FIR)/(RFD \times BW \times AT) \times 10^{-3}$$

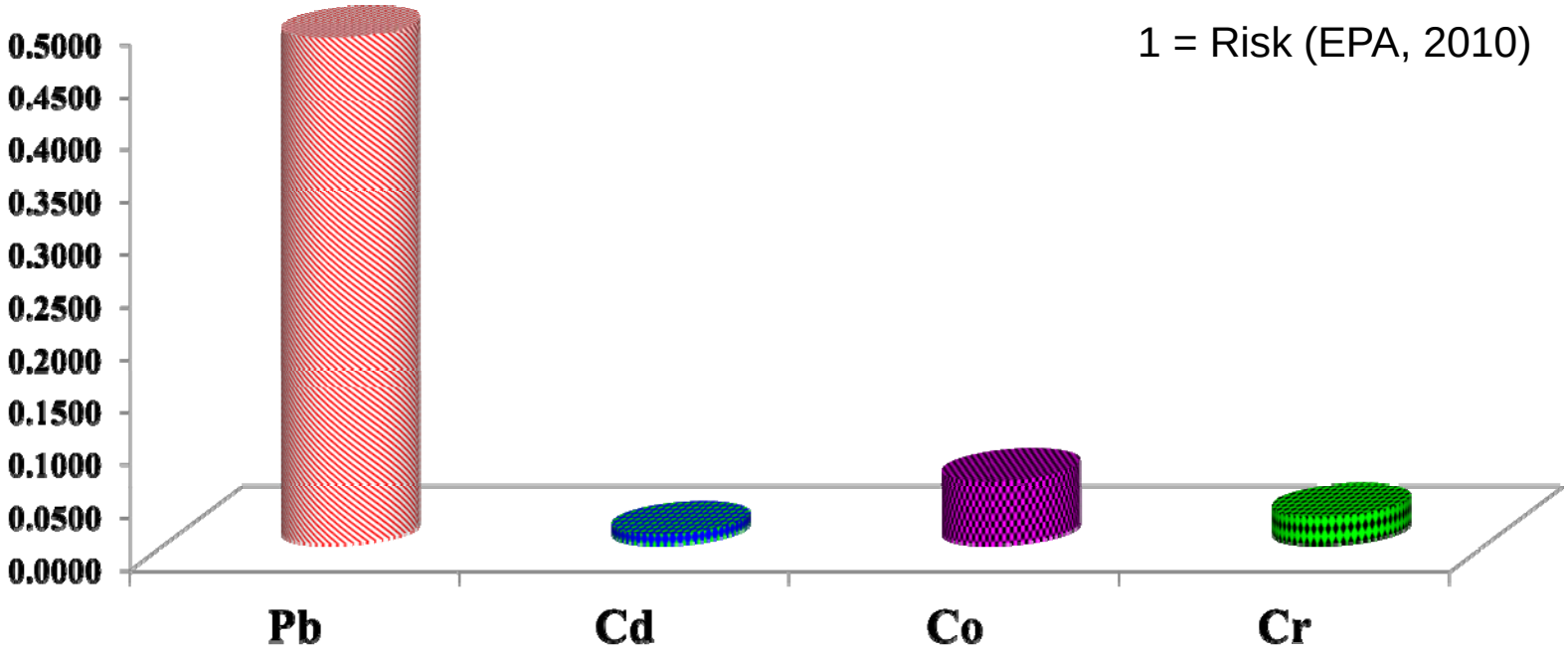
- **Total target hazard quotient**

  $$TTHQ = THQ_{Pb} + THQ_{Cd} + THQ_{Co} + THQ_{C}$$

The calculation of Incremental lifetime cancer risk (ILCR) in duck egg by absorption of carcinogenic heavy metals according to USEPA.



The calculation of Target Hazard Quotient (THQ) towards each heavy metals in duck egg base on the duration of human exposure (70 years)



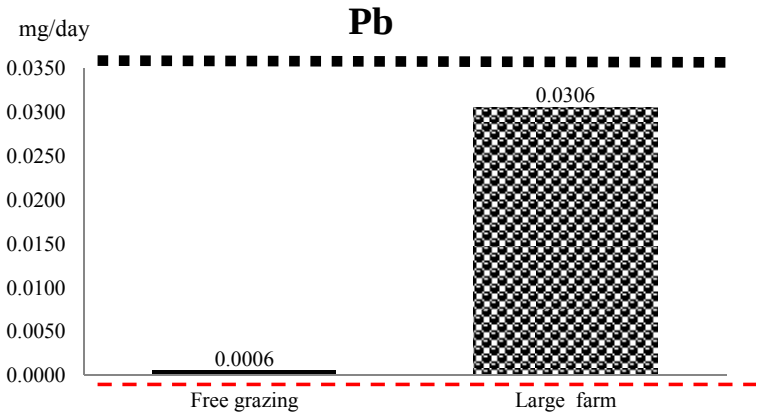
The estimated THQ of duck egg consumption in 70 year olds of Thai population found that Pb>Co>Cr>Cd, respectively.

However the TTHQ (0.58) was below the standard limit of 1 that means it is no risk.

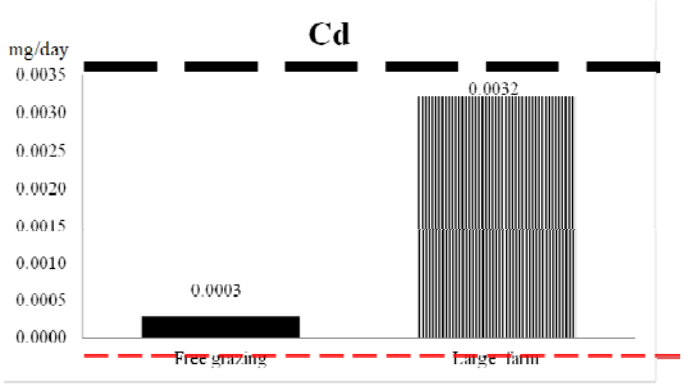
Comparison of Zinc, Pb, Cd, Co, Mn, Fe, Cr and Cu levels in duck meat, liver and intestines from free grazing and large scale farms (mg/kg dry weight)

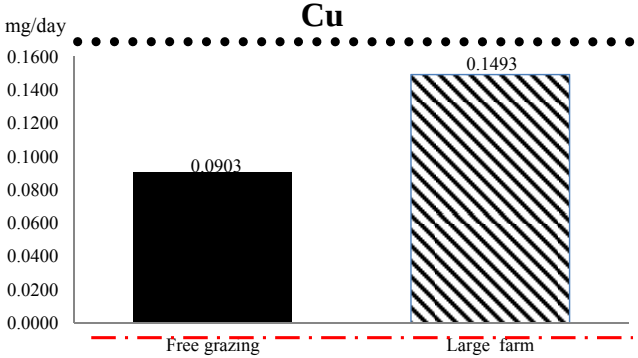
Samples	Variables		Zn	Pb	Cd	Co	Mn	Fe	Cr	Cu
Meat	Free grazing	Min-Max level	11.66-75.91	ND-1.13	ND-0.13	ND-ND	ND-2.97	71.56-248.02	ND-0.31	1.56-19.02
		Mean+SD	34.67±13.13	0.06 ±0.21	0.03 ±0.04	ND	0.20±0.50	129.12 ±47.76	0.01±0.05	9.24±4.28
	Large scale farm	Min-Max level	13.09-94.03	0.55- 6.37	0.02-0.71	ND-0.49	ND-2.09	31.68-297.14	ND-2.02	8.12-21.58
		Mean+SD	47.28±16.80**	3.13±1.13**	0.33±0.14**	0.14±0.10	0.69±0.66**	154.45±68.85*	0.29±0.44**	15.28±3.37**
	NMPHT		-	1.0	-	-	-	-	-	-
		Free grazing	-	2.86%(1/35)	-	-	-	-	-	-
		Large farm	-	92.73%(51/55)	-	-	-	-	-	-
	EC		-	0.1	0.050	-	-	-	-	-
		Free grazing	-	14.29% (5/35)	34.29%(12/35)	-	-	-	-	-
		Large fram	-	100% (55/55)	96.36% (53/55)	-	-	-	-	-
	FAO/WHO		-	0.1	0.050	-	-	-	-	-
		Free grazing	-	14.29% (5/35)	34.29%(12/35)	-	-	-	-	-
		Large farm	-	100% (55/55)	96.36% (53/55)	-	-	-	-	-
Liver	Free grazing	Min-Max level	69.55-214.95	ND-10.42	ND-3.24	ND-1.05	4.20-21.88	185.40-5307.43	ND-3.59	7.53-240.34
		Mean+SD	116.13±39.36	3.01±2.77	0.93 ±0.85*	0.27±0.26	11.23 ±4.66	1162.91 ±966.49	0.36±0.82	92.52 ±61.64
	Large farm	Min-Max level	91.25-542.02	0.23-6.69	ND-1.06	ND-1.32	7.98-55.90	118.54-3329.19	ND-2.25	93.71-516.42
		Mean+SD	214.10±91.27**	3.14±1.49	0.48±0.23	0.44±0.27*	21.41±11.16**	835.16±573.24	0.52±0.59*	239.08±85.77**
	NMPHT		-	1.0	-	-	-	-	-	-
		Free grazing	-	64.71%(22/34)	-	-	-	-	-	-
		Large farm	-	94/44%(51/54)	-	-	-	-	-	-
	EC		-	0.10	0.50	-	-	-	-	-
		Free grazing	-	70.58%(24/34)	67.64(23/34)	-	-	-	-	-
		Large farm	-	100%(54/54)	42.59(31/54)	-	-	-	-	-
	FAO/WHO		-	0.10	0.50	-	-	-	-	-
		Free grazing	-	70.58%(24/34)	67.64%(23/34)	-	-	-	-	-
		Large farm	-	100%(54/54)	42.59%(31/54)	-	-	-	-	-
Intestines	Free grazing	Min-Max level	39.80-132.00	0.95-3.94	0.05-1.53	ND-0.82	4.29-64.80	86.09-3322.11	0.13-7.61	1.81-14.84
		Mean+SD	70.79±19.98	2.07±0.76**	0.28±0.33**	0.23±0.21	20.40±18.31	358.73±557.70**	1.55±1.54**	6.36±2.97
	Large farm	Min-Max level	48.08-185.51	0.36-3.71	ND-0.28	ND-2.22	3.66-104.45	33.31-132.03	0.02-2.43	1.63-17.83
		Mean+SD	86.77±26.67**	1.44±0.64	0.08±0.09	0.51±0.63	36.46±26.30**	71.43±22.56	0.61±0.46	7.84±4.40
	NMPHT		-	1.0	-	-	-	-	-	-
		Free grazing	-	97.06%(33/34)	-	-	-	-	-	-
		Large farm	-	76%(38/50)	-	-	-	-	-	-

ND = Not Detected, - No standard limit, * = Significant difference at $P < 0.05$, ** = Significant difference at $P < 0.001$, NMPHT= Notification of Ministry of Public Health No. 98(B.E.2529) of Thailand [77], EC= (EC) COMMISSION REGULATION No .1881/2006 [75], FAO/ WHO =FAO/WHO 2002 and Codex Alimentarius



A WHO provisional tolerated daily intakes in mg/day per capita of Pb(0.429 mg/day)
JECFA (2011) (0.003 mg/kg body weight/day)



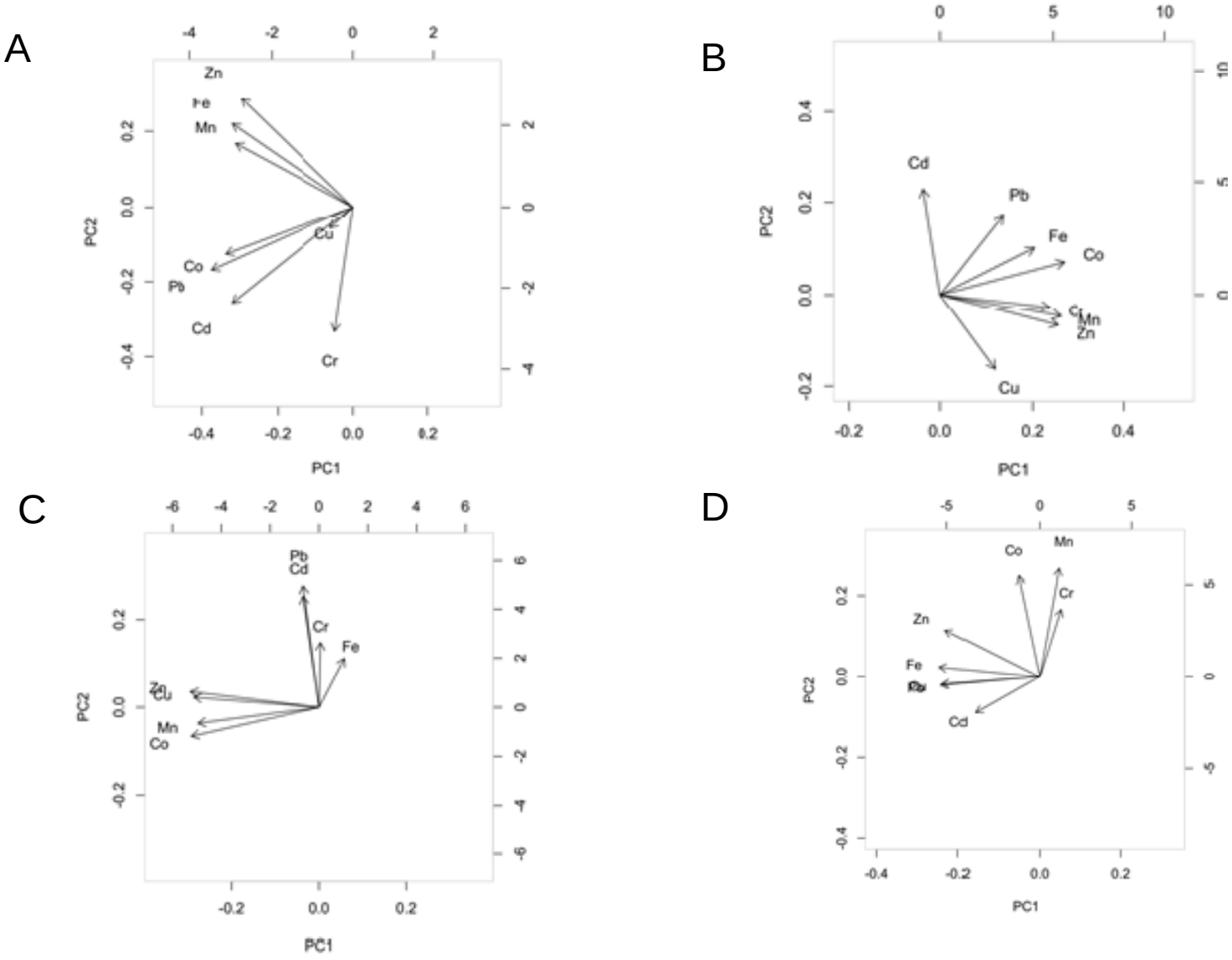


B WHO provisional tolerated daily intakes mg/day per capita of Cd (0.05-0.071 mg/day)
Nogawa et al. (1989) and ATSDR (1999) (0.0002 mg/kg/day)

C Recommended Daily Intake (RDI) mg/day of Cu (2 mg/day)
ATSRD (2004) (0.01 mg/ kg/day)

The calculation of average daily intake based on duck meat consumption according to the WHO provisional tolerated daily intake and RDI. This shows an average daily intake of Pb, Cd and Cu presented at lower levels than the standard limit.

PCA plot showing metals loadings on components from meat (A), liver (B), intestine (C) and combined between liver and intestine (D)



Highlight

- The Cd levels in duck liver from free grazing farms was higher than in liver samples from large scale farms at $P<0.05$.
- • The highest levels of Zn, Pb, Cd, Fe and Cu contamination were found in the liver more than other organs.
- • The consumer may incur health risks from consumption of duck meat high in Pb, Cd and Cu concentration from both types of farms, particularly focused in duck meat from large scale duck farms.

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Health Risk Contamination of Heavy Metals in Yolk and Albumen of Duck Eggs Collected in Central and Western Thailand

P. Aendo[1] · R. Netvichian[2] · S. Tippayalak[3] · A. Sanguankiat[1,3] · T. Khuntamoon[1,3] · T. Songserm[1,4] · P. Tulayakul[1,3]

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Heavy Metal (Cd and Pb) and Aflatoxin Contamination in Tissues and Eggs from Free Grazing Ducks and Their Environment in Central Thailand

P. Tulayakul[1,2] · R. Mingkhwan[3] · H. Hananantachai[3] · R. Netvichian[4] · S. Khaodhiar[4] · T. Songserm[2,5]

ELSEVIER

Ecotoxicology and Environmental Safety

Volume 161, 15 October 2018, Pages 691-698

Comparison of zinc, lead, cadmium, cobalt, manganese, iron, chromium and copper in duck eggs from three duck farm systems in Central and Western, Thailand

P. Aendo [a, c], R. Netvichian [b], S. Viriyarampa [a], T. Songserm [c, d], P. Tulayakul [a, c]

Mahidol University
Faculty of Tropical Medicine

ENVIRONMENTAL ENGINEERING
CHULA ENGINEERING
Foundation toward Innovation

Center for Duck Health Science, Kasetsart University

Thank you very much