

Developmental Uptake of Radiolabeled 3,3',4,4'-Tetrachlorobiphenyl (PCB 77) into Japanese Quail Egg Compartments and Embryos

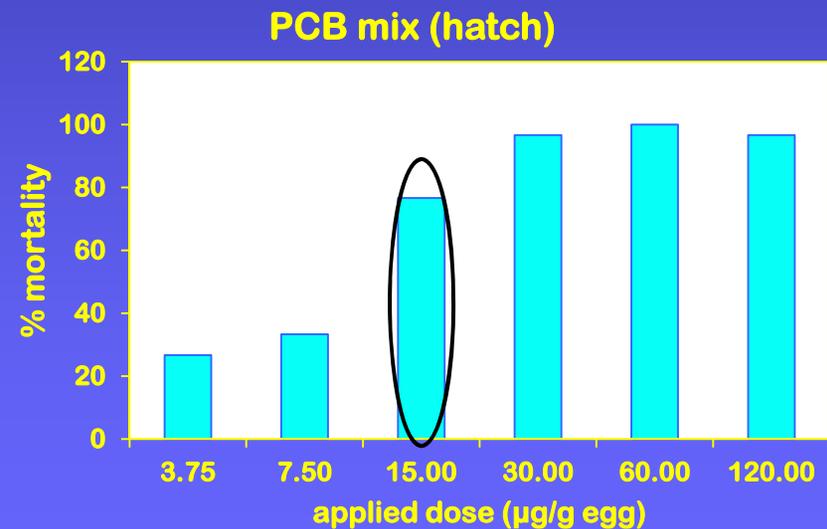
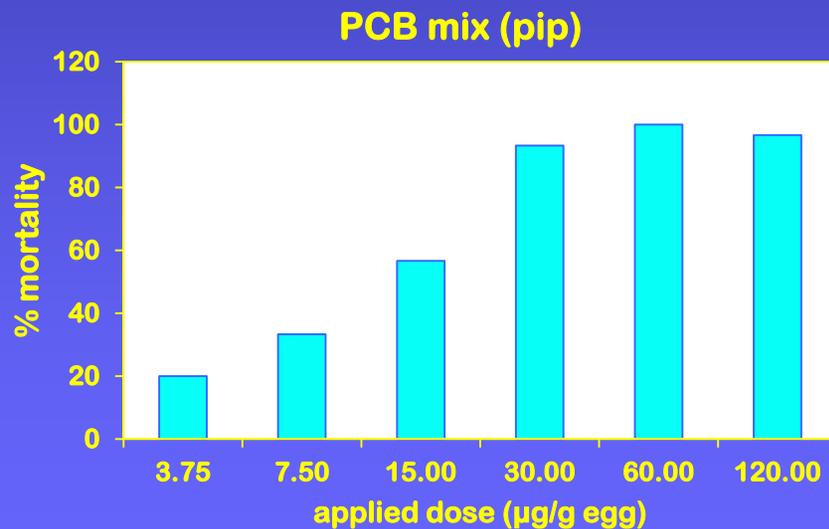
Karen Dean and Mary Ann Ottinger

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K.M. DEAN and M.A. OTTINGER

How well are PCBs delivered to the embryo following egg injection?

- Unlike maternal deposition uptake by the embryo is dependent on:
 - Injection site
 - Solubility in egg components
 - Timing of injection



Does the quail strain matter?

Randomly Bred colony



- smaller egg volume (10g)
- only white eggs
- heterogeneous colony, not used for food production
- lower fertility and hatching success (~80%)
- eggs available all year but retains seasonality

treatment	Jun 21 –Dec 21		Dec 21-Jun 21	
	% hatch	n	% hatch	n
corn oil	56.25	16	75.00	24
fatty acid mix	56.25	16	80.00	25
untreated	56.25	16	78.26	46
sham	58.82	17	76.60	47

Embryonic Day	↑ E ₂ & androgen							Hatch
	1	3	5	8	10	12	15	
Event	cell differentiation	gonadal development		GnRH-I cells migrate		behavior differentiation	HPG axis function	piping
Effects of Treatments		↑ (alter gonadal differentiation)		↑ (affect GnRH-I system)		↑ (sexual differentiation of hypothalamic neural systems)		

- ED 3-5: yolk cells continue to enlarge and differentiate
- ED 10: thyroid axis is developing
- ED 12: most of albumen absorbed by embryo
- ED 13: sexual development has occurred, brain development is ongoing
- ED16: initiation of pipping, higher energy expenditure, use of yolk increases
- Hatch: massive energy expenditure, HPA and HPT axes surge, yolk use high

Sub-Lethal Effects of Embryonic PCB Exposure

- **Deformities**
- **Cardiovascular impact**
- **Liver**
- **Endocrine function**
- **Immune function**
- **Gene expression**
- **Neuroendocrine (GnRH and monoamines)**
- **Oxidative damage**



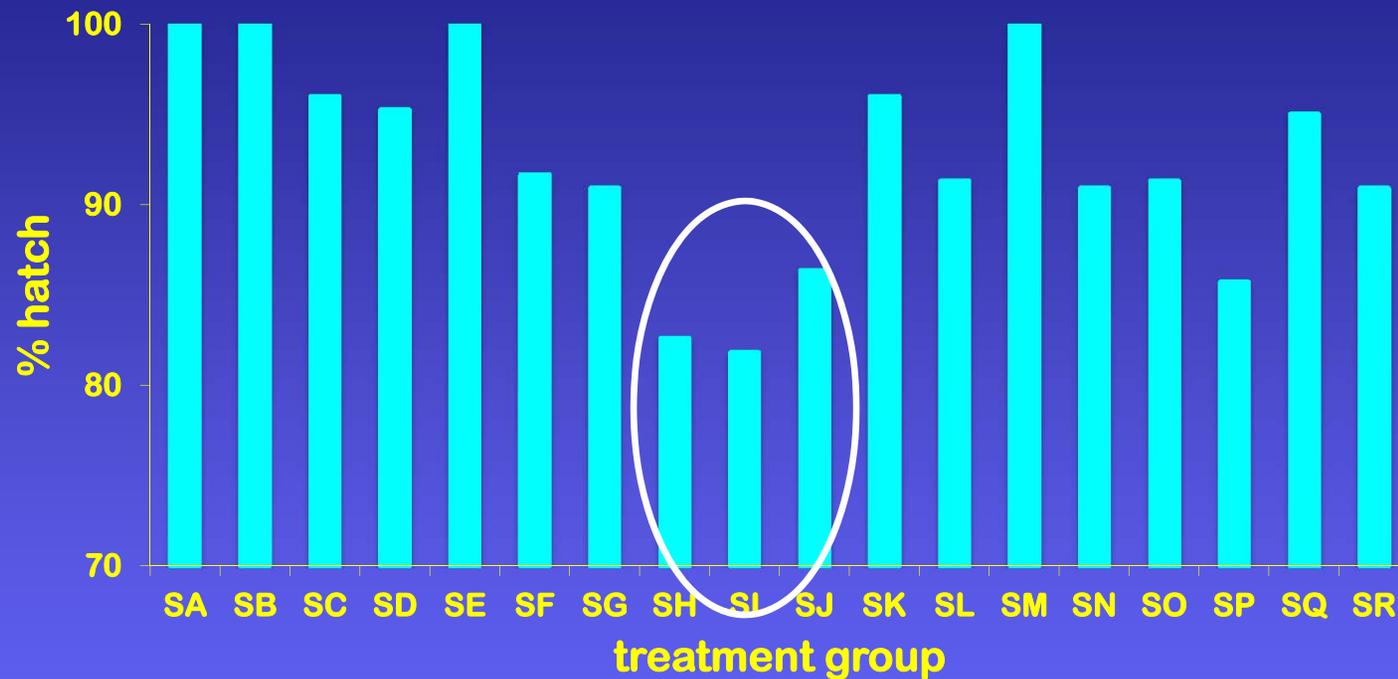
Egg injection protocol development

- **When do we inject?**
- **How do we inject?**
- **Where?**
- **What volume?**
- **What vehicle?**
- **How do we seal the egg?**
- **How do we incubate the egg?**
- **How do we make sure that we deliver compound?**
- **ED 0 or ED 3**
- **needle or Dremel and pipette**
- **air cell or albumen**
- **0.1 or 0.4 μ l/g of egg**
- **vegetable oil, triolein or fatty acid mix**
- **tissue glue or paraffin**
- **vertically or horizontally**

Sham injection Protocol

Sham group	Injection technique	Injection site	Sealant	Incubation position		
SA	N/A	N/A	N/A	vertical		
SB	N/A	N/A	N/A	horizontal		
SC	needle	air cell	Super glue	vertical		
SD			horizontal			
SE			paraffin	vertical		
SF			horizontal			
SG		side	Super glue	vertical		
SH			horizontal			
SI			paraffin	vertical		
SJ			horizontal			
SK			Dremel	air cell	Super glue	vertical
SL					horizontal	
SM	paraffin	vertical				
SN	horizontal					
SO	side	Super glue		vertical		
SP		horizontal				
SQ		paraffin		vertical		
SR		horizontal				

Sham Injection Results

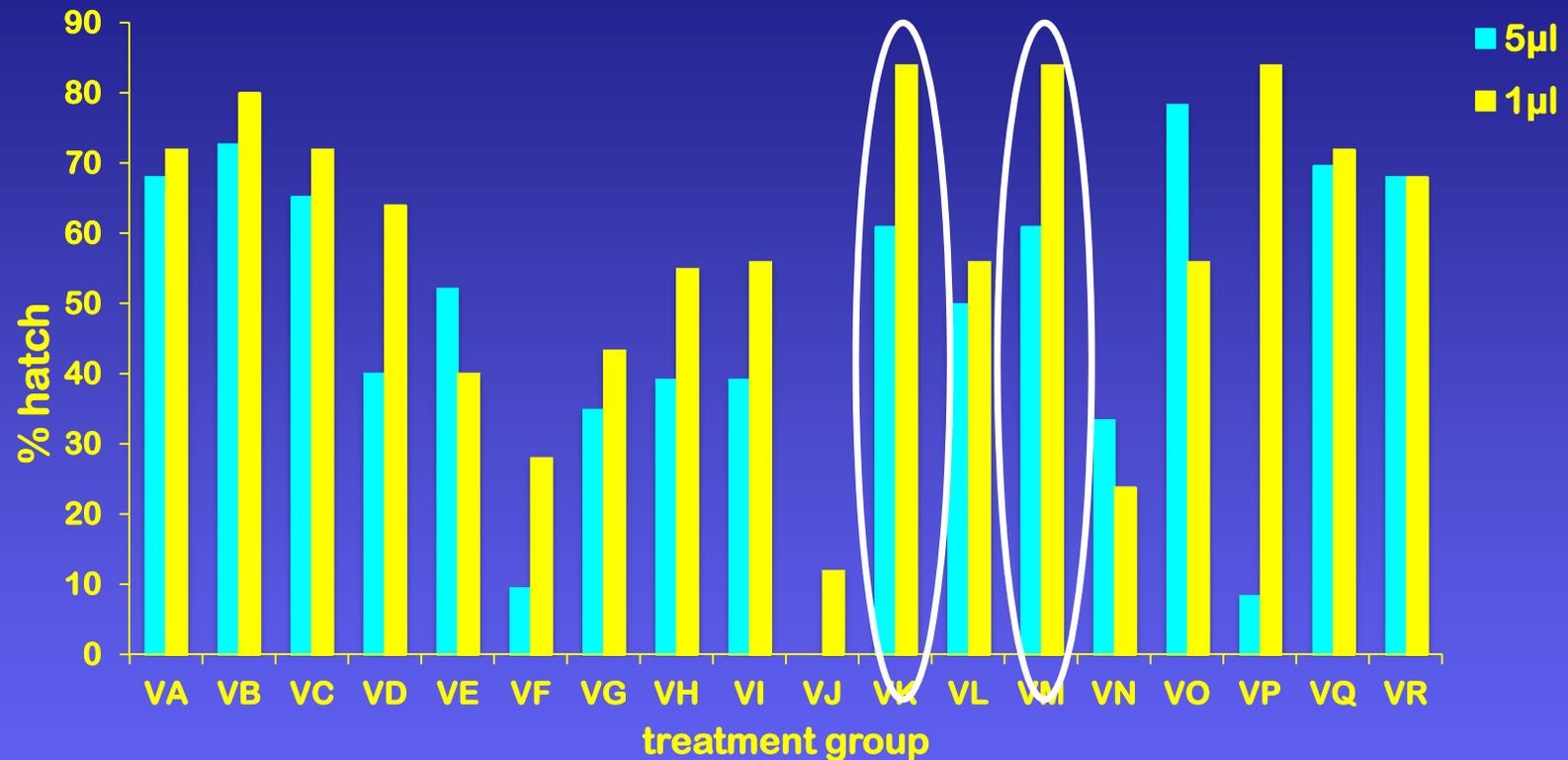


Injection site and incubation position have an effect, but sealant and injection method, did not affect in early embryo mortality

Vehicle injections: performed at 1.0 and 5.0 μ l

Vehicle group	Vehicle	Injection site	Incubation position
VA	N/A	N/A	vertical
VB	N/A	N/A	horizontal
VC	corn oil	air cell	vertical
VD			horizontal
VE		side	vertical
VF			horizontal
VG	triolein	air cell	vertical
VH			horizontal
VI		side	vertical
VJ			horizontal
VK	JQE yolk FA mix	air cell	vertical
VL			horizontal
VM		side	vertical
VN			horizontal
VO	sham	air cell	vertical
VP			horizontal
VQ		side	vertical
VR			horizontal

Vehicle and Delivery

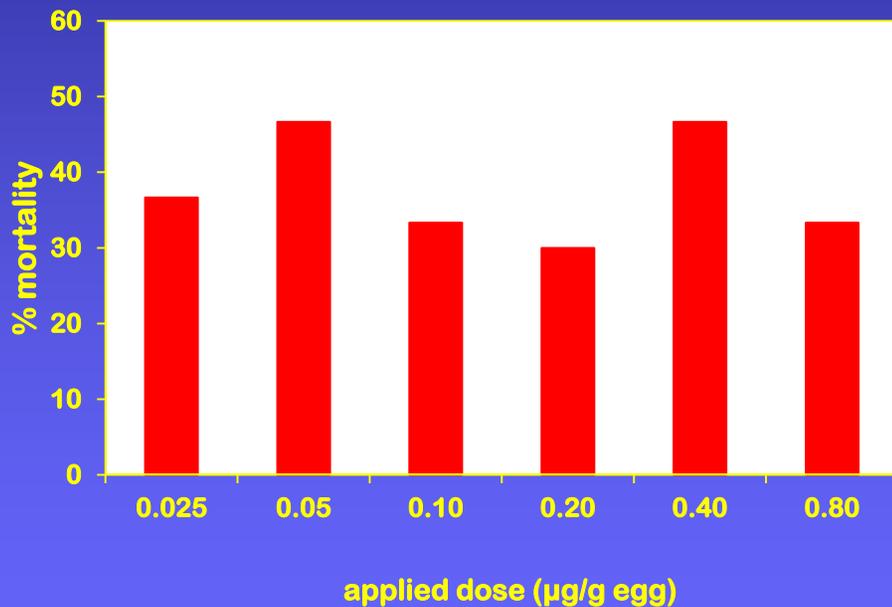


**Optimal conditions: 1µl of fatty acid mix in the air cell
or albumen with egg incubated vertically**

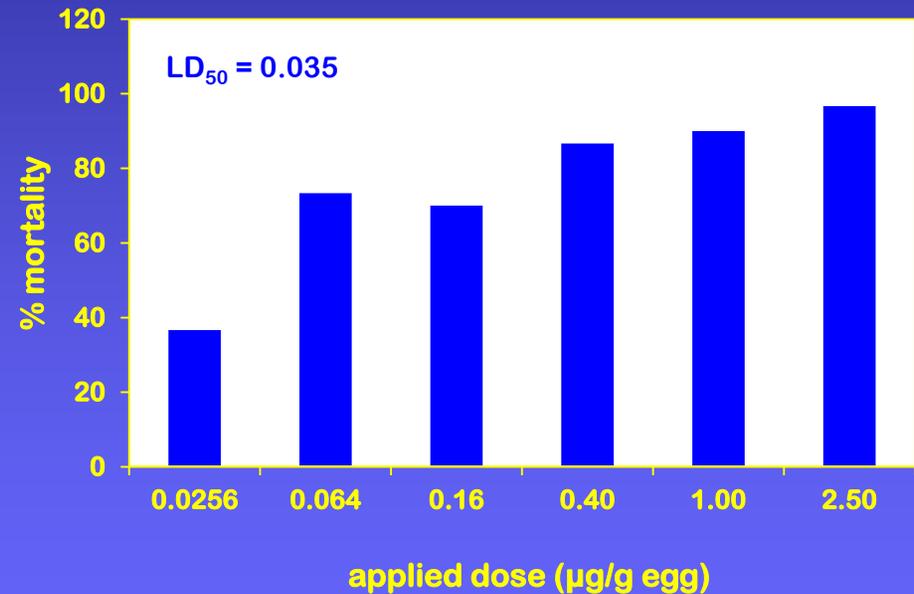
¹⁴C PCB 77

- Unlike the other PCBs tested, PCB 77 does not produce a clear lethality curve in this Japanese quail strain

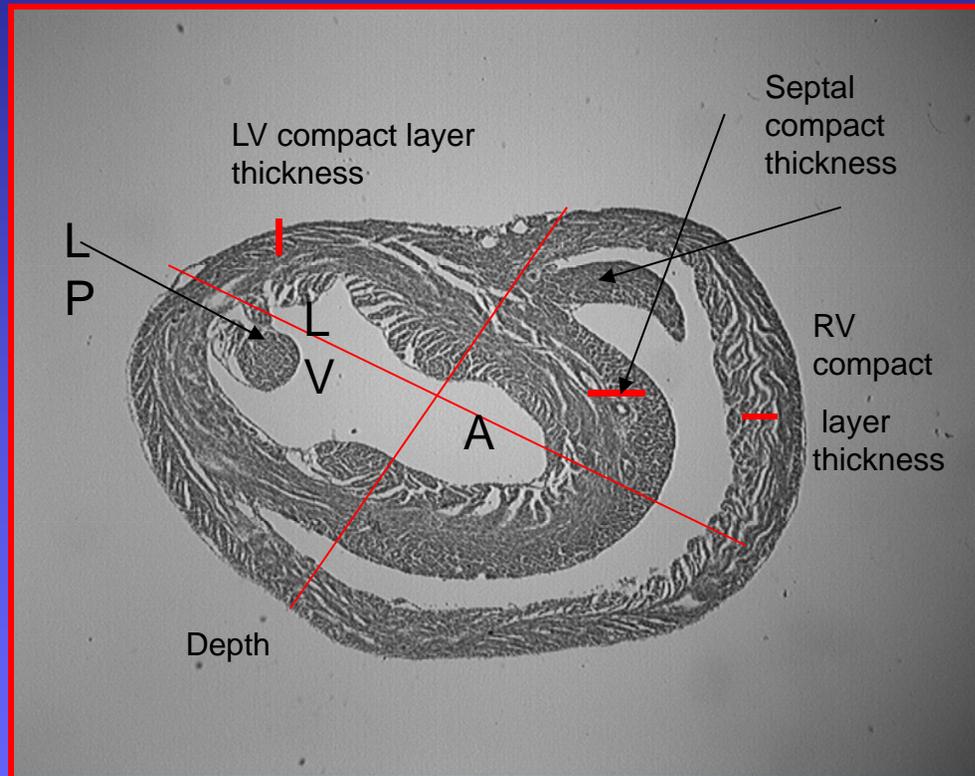
PCB 77 (hatch)



PCB 126 (hatch)



Avian Egg Bioassay for Understanding Sub-Lethal Effects of PCBs

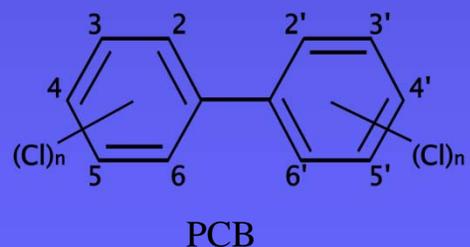


- Tree swallows eggs treated with $1\mu\text{g/g}$ egg PCB 77 at 18% of embryonic development lack a compact layer
- Chickens treated with a PCB mix show similar cardiac deformities



Effects of PCBs on Early Embryonic Development

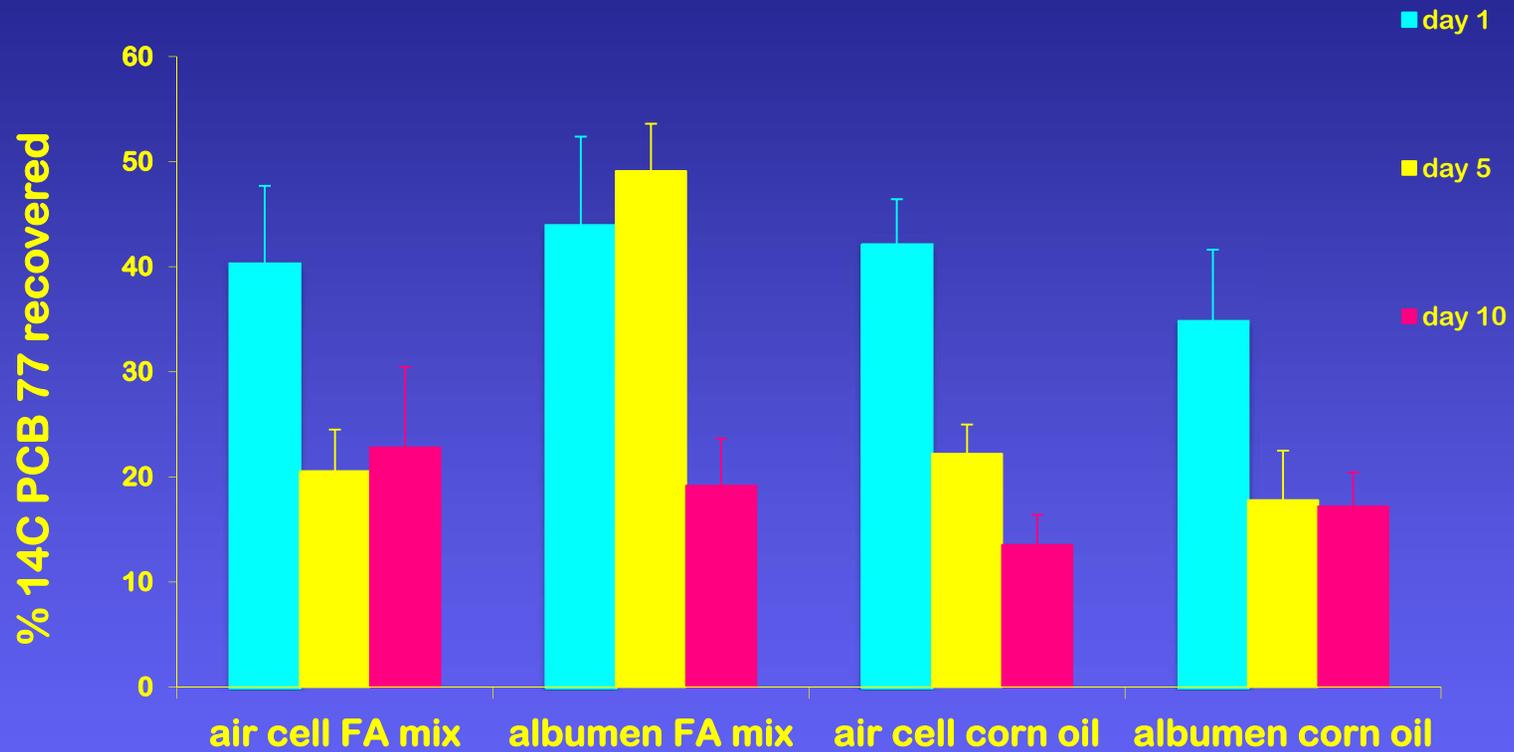
- 0.1 μ l of 14 C PCB 77 per gram of egg equivalent to 40-60ng/g egg
- ED0: Inject early to determine if how cardiac abnormalities could occur
- Early stage of development to determine if there is transfer to yolk and embryo across membranes



¹⁴C PCB 77 Diffusability: How much PCB gets to the embryo?

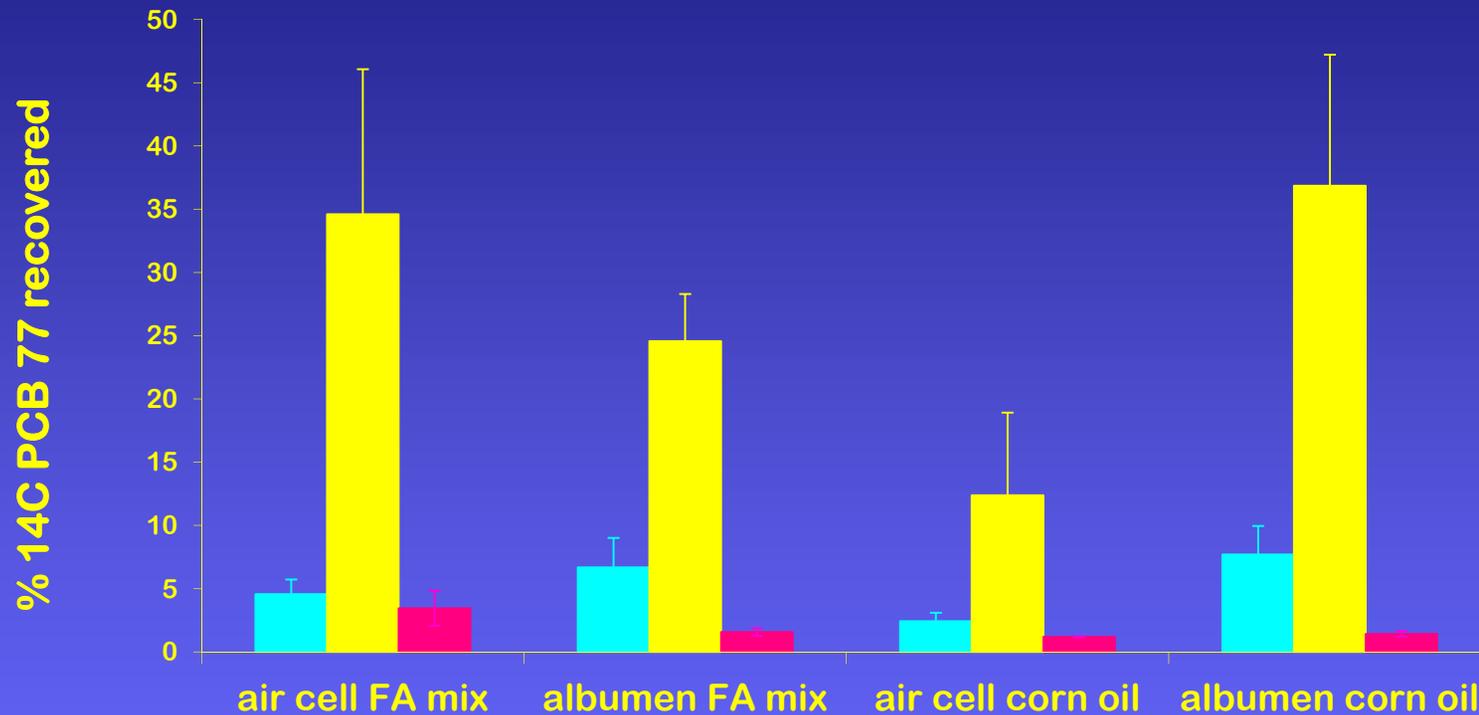
Group	vehicle	injection site	volume (μl)	collection time
HA	JQ yolk fatty acid mix	air cell	1	24h
HB				5 days
HC				10 days
HD		albumen		24h
HE				5 days
HF				10 days
HG	corn oil	air cell	1	24h
HH				5 days
HI				10 days
HJ		albumen		24h
HK				5 days
HL				10 days
HM	untreated	n/a	0	24h
HN				5 days
HO				10 days

Shell Content



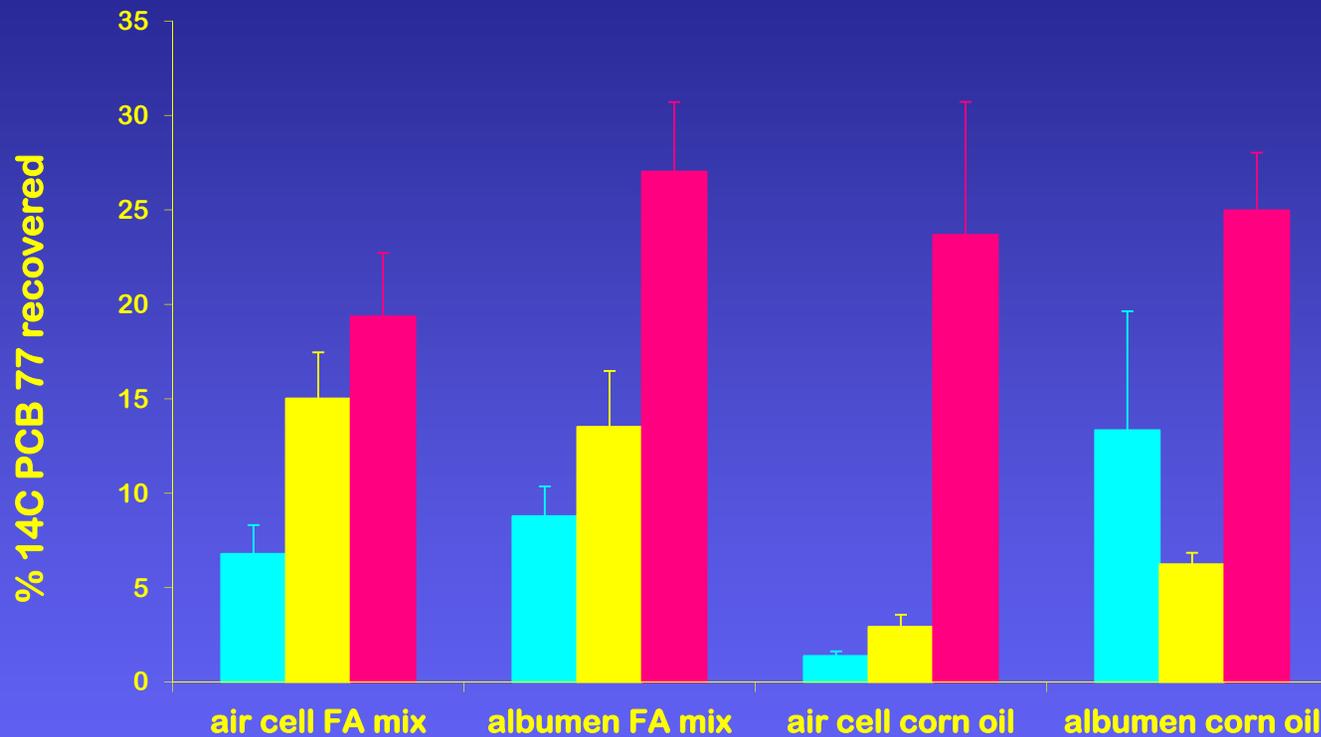
transfer across membranes continues

Albumen content



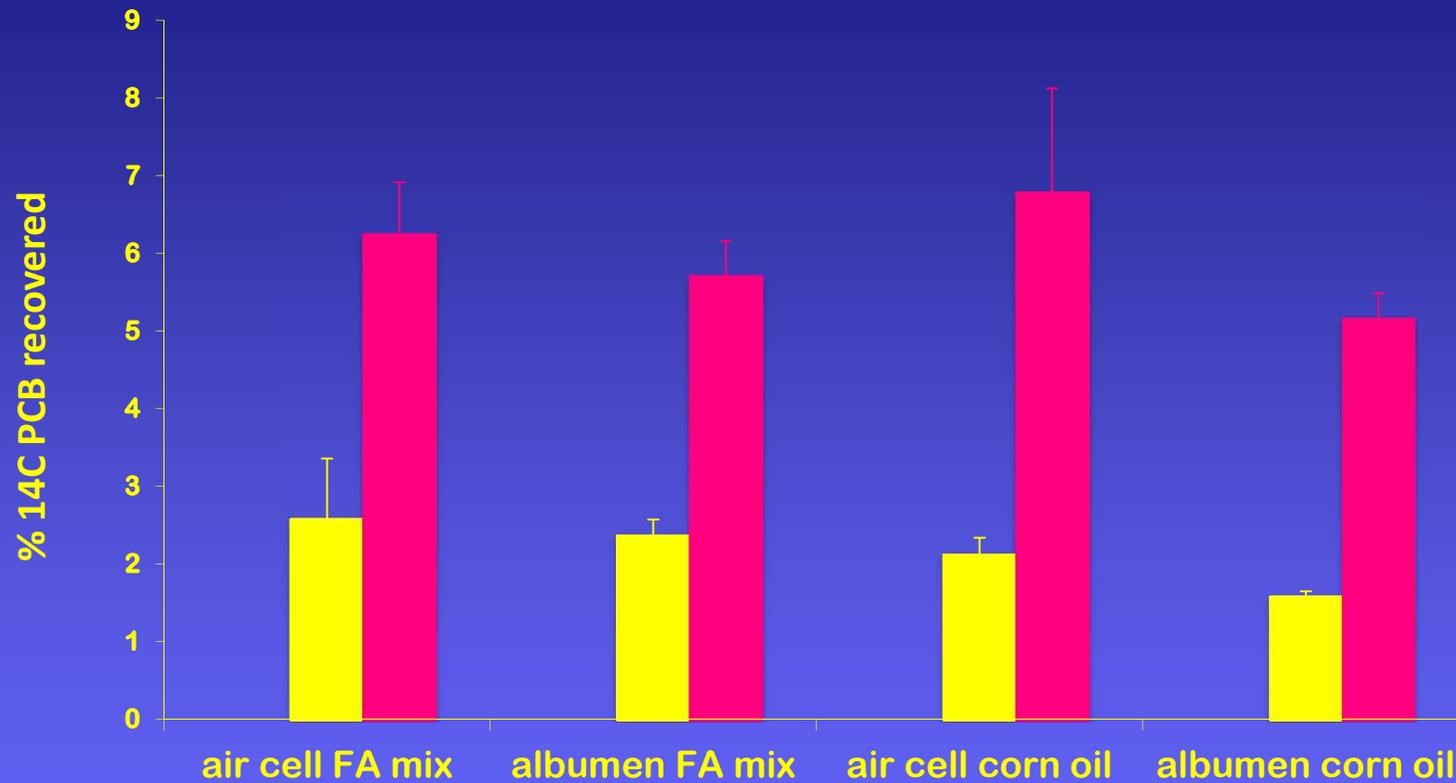
Transitory increase in ¹⁴C PCB 77 albumen levels

Yolk Content



¹⁴C PCB 77 increases in yolk

Embryo Content



¹⁴C PCB 77 reaches the embryo

Diffusion of ^{14}C PCB 77 into Japanese quail egg

- Regardless of application to air cell or albumen, some ^{14}C PCB 77 is retained in the shell/membrane
- Gradual diffusion throughout development
- PCB migrates from the shell/membrane, transits through the albumen and reaches the yolk and embryo
 - shell/membrane decreases indicate possible a rate-limiting step
 - albumen peaks at ED5 and drops off by ED10
 - yolk increases from from ED1 to ED10
 - embryo only contain 2% by ED5 and 6% by ED10
- Maternal deposition occurs directly into yolk
- Uptake in our study and in others indicate low uptake early in development; increasing with embryonic development
- Highest potential exposure is late in incubation and early post hatch when the embryo is dependent on yolk for nutrients

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