

# Avian Dosing Study:

## an avian two-generation test development activity

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# SCIENTIFIC BASIS OF AVIAN TWO-GENERATION TESTS

- Hormonal control of sexual differentiation in birds differs from that of mammals
- Oviparity in birds affects retention and transfer of compounds
- Current protocols
  - are not sufficiently robust to define all reproductive or developmental effects from endocrine disrupting chemicals
  - are not designed to determine long-term effects of *in ovo* exposure or fitness of offspring
  - do not assess effects at all 4 critical life stages that could be sensitive to endocrine disruption

# Exposure Considerations

- Exposure of parental (P1) generation
  - Pre-egg laying
    - Effects on sexual maturation possible
    - Bioaccumulation of test substance
  - Post-initiation of laying (proven breeders)
    - Statistical advantages
      - removing nonproductive birds before exposure
      - Use pretreatment measures as covariates
    - Reduction in cost from reduced exposure period
    - Rapidity in manifestation of reproductive effects observable

# Exposure Considerations (cont.)

- Exposure of offspring (F1) of parents
  - Exposure from hatch through egg-laying
    - Worse case scenario
    - Allows observation of effects at all life stages
  - No exposure (*in ovo* exposure only)
    - Avoids masking of endocrine-mediated effects by high mortality of chicks from direct toxicity
- F2 chicks not exposed

# Exposure Considerations (cont.)

- Optimization of exposure scenarios for P1 and F1
  - Intended to maximize attributes, minimize disadvantages
    - Expose maximum number of reproductive processes
    - Expose the maximum number of life stages
    - Increase power of test
    - Not mask endocrine-related effects/not confound interpretation of results
    - Worst-case environmental exposure
    - Time and cost-effectiveness
- Dosing Study

# Avian Dosing Study

Work Performed by

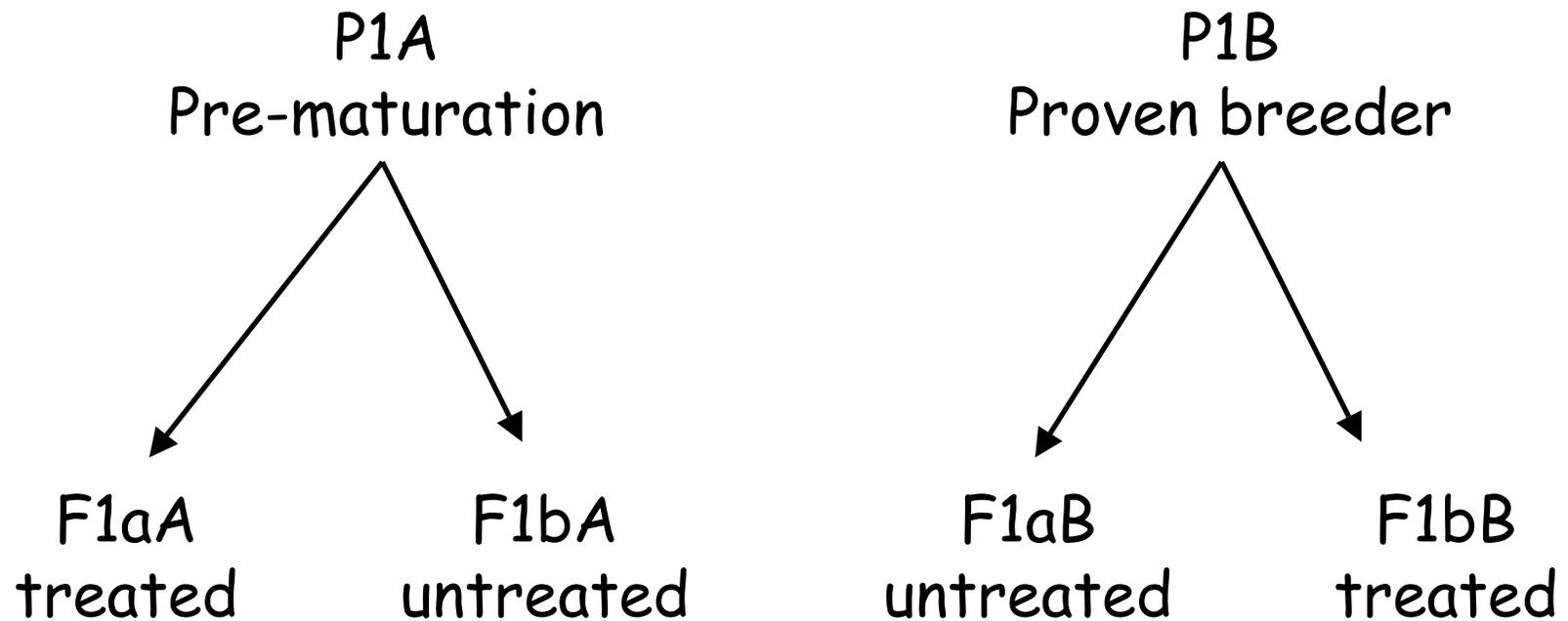


On behalf of the United States Environmental Protection  
Agency EPA CONTRACT NUMBER 68-W-01-023

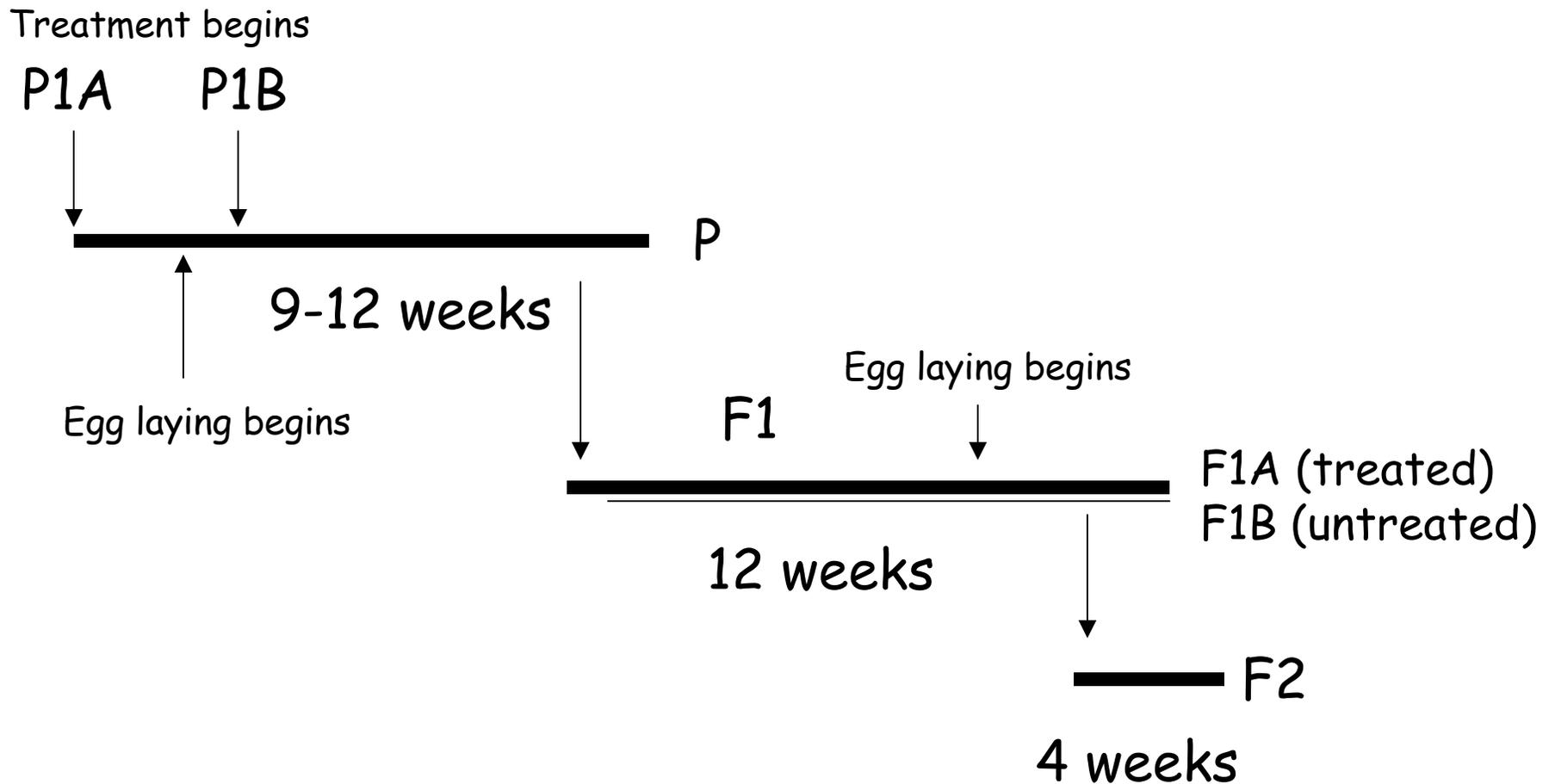
## Objectives

- Compare treatment-related responses between the two P1 exposure scenarios - pre-breeding vs. post-breeding groups
- Compare treatment-related responses between the two F1 exposure scenarios - exposed vs. unexposed

# Study Design



# Avian Dosing Study Schematic



# Endpoints

- Fitness Endpoints:
  - Egg production
  - Fertility
  - Eggshell integrity
  - Embryo viability
  - Hatchability
  - Sex ratio
  - Chick health
  - Signs of toxicity

# Endpoints (cont.)

- Endocrine Endpoints:

## Gross morphology & histopathology

- Organ/gland weights/bone length(chicks)
- Organ/gland histology
- Spermatid counts & morphology
- Gross anomalies

## Developmental landmarks

- Feather dimorphism
- Cloacal gland size
- Sexual maturation/behavior

## Plasma and fecal/urate hormones

- Steroid hormones
- Thyroid hormones

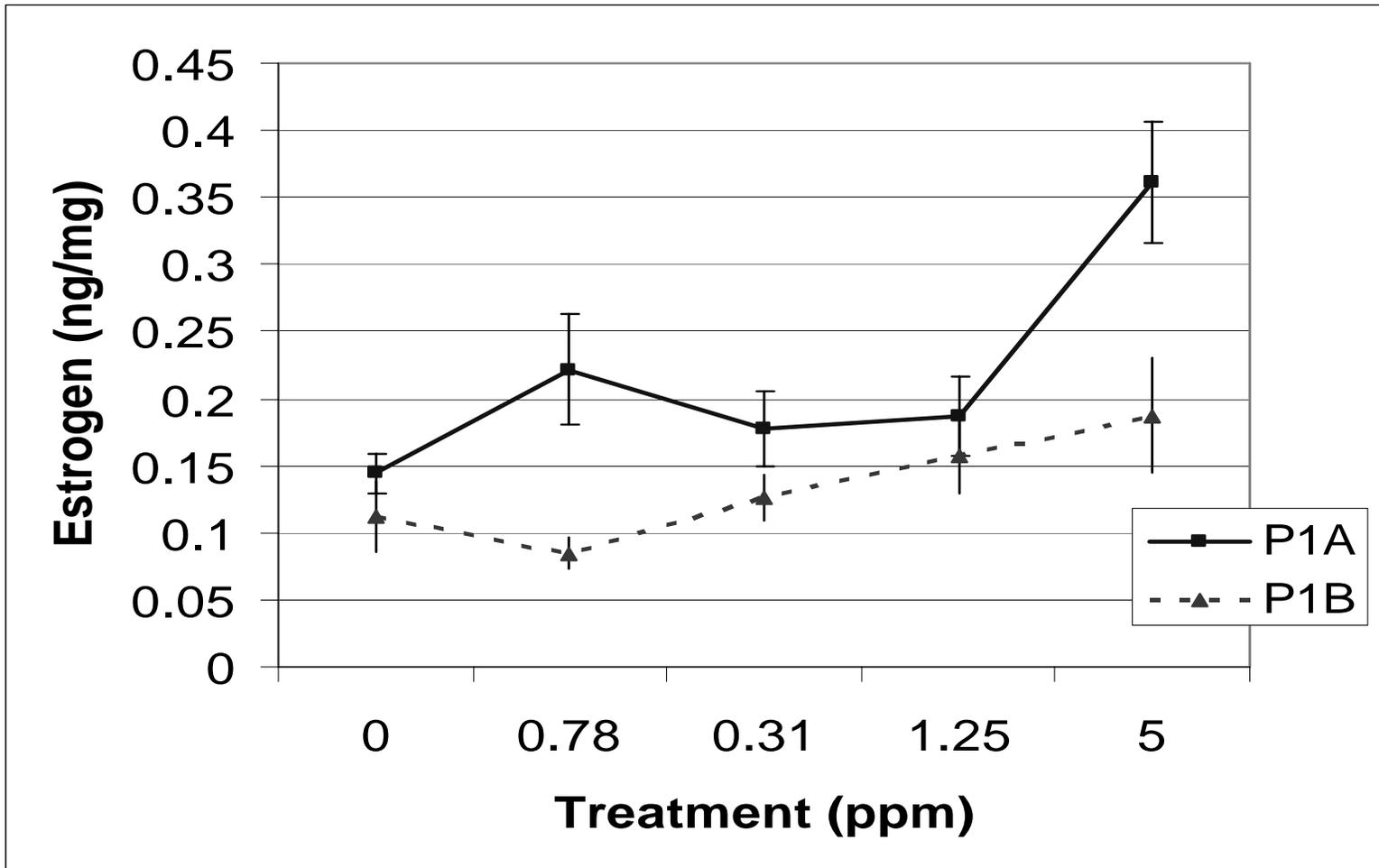
# Range Finding Trial

- Test compound:  $17\beta$ -estradiol
- Treatment levels: 1 ppm, 10 ppm, and 100 ppm
- 3 pairs per level
- Treated for 14 days egg-laying
- Evaluated for survival, body weight, egg production, embryo viability, hatchling survival
- Treatment levels selected:  
0 , 0.078, 0.31, 1.25 and 5 ppm

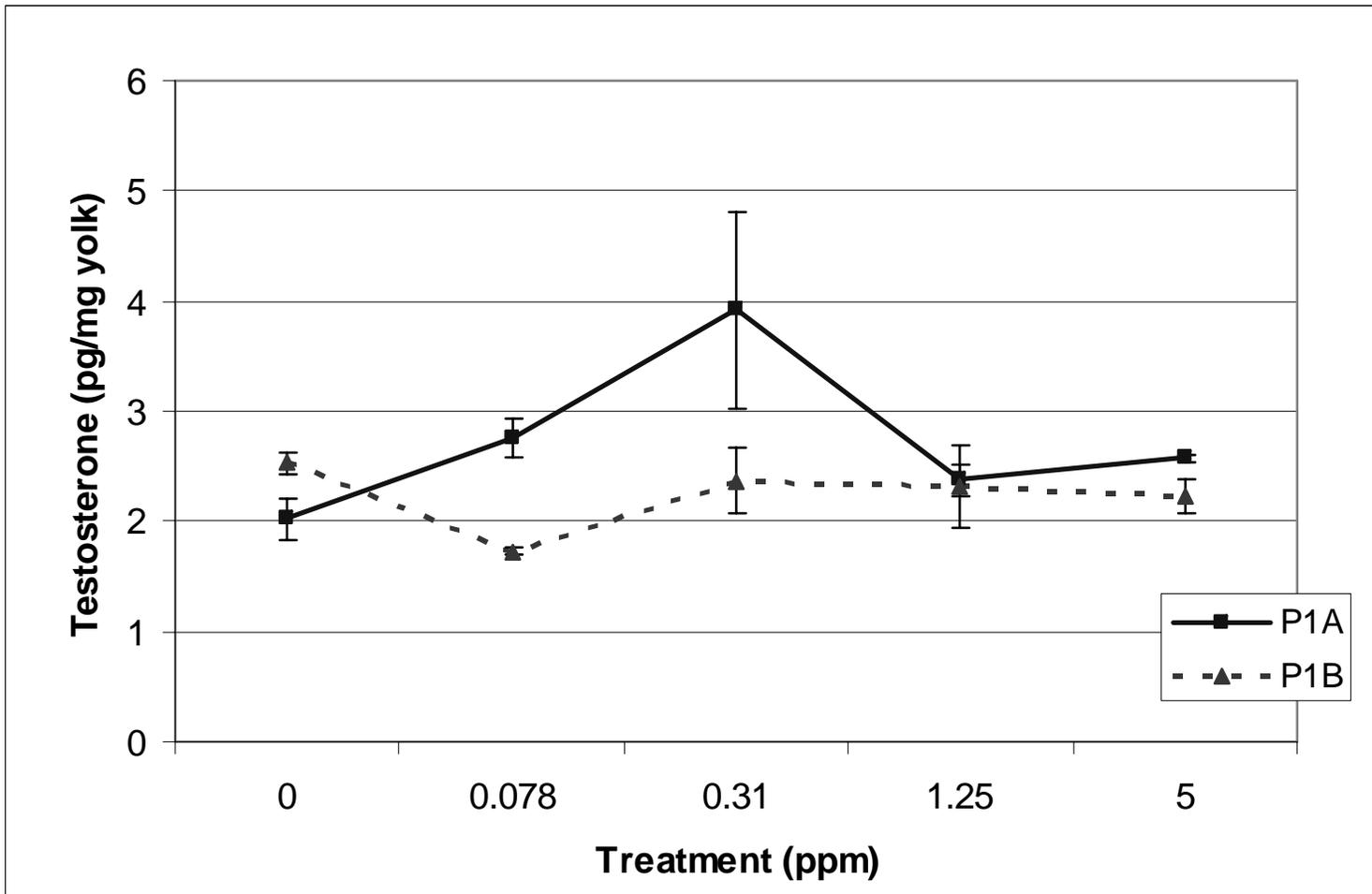
# RESULTS

- Variability in data high; aggression of females to introduced males resulted in some deaths; male aggression also observed; loss of mates and probable stress contributed to observed variability

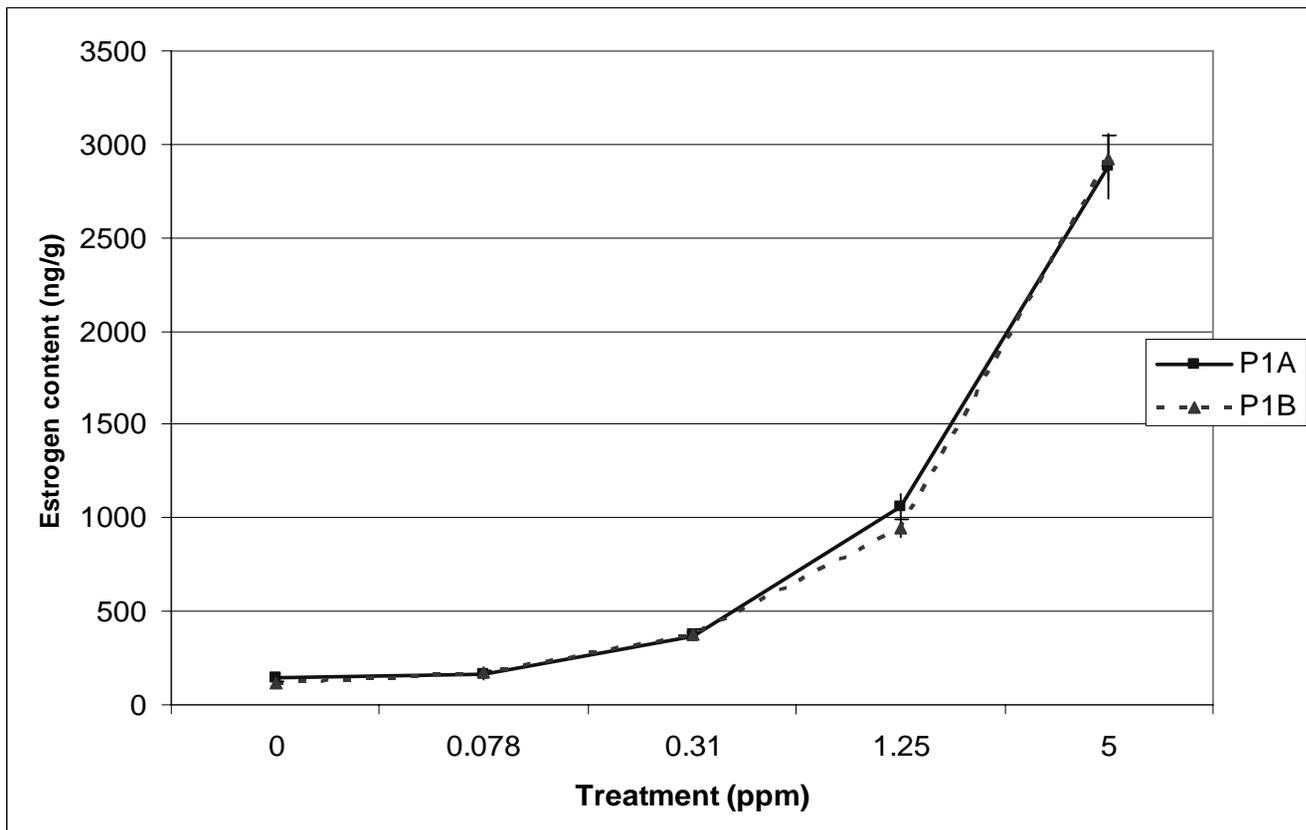
# Egg estrogen content for P1 generation



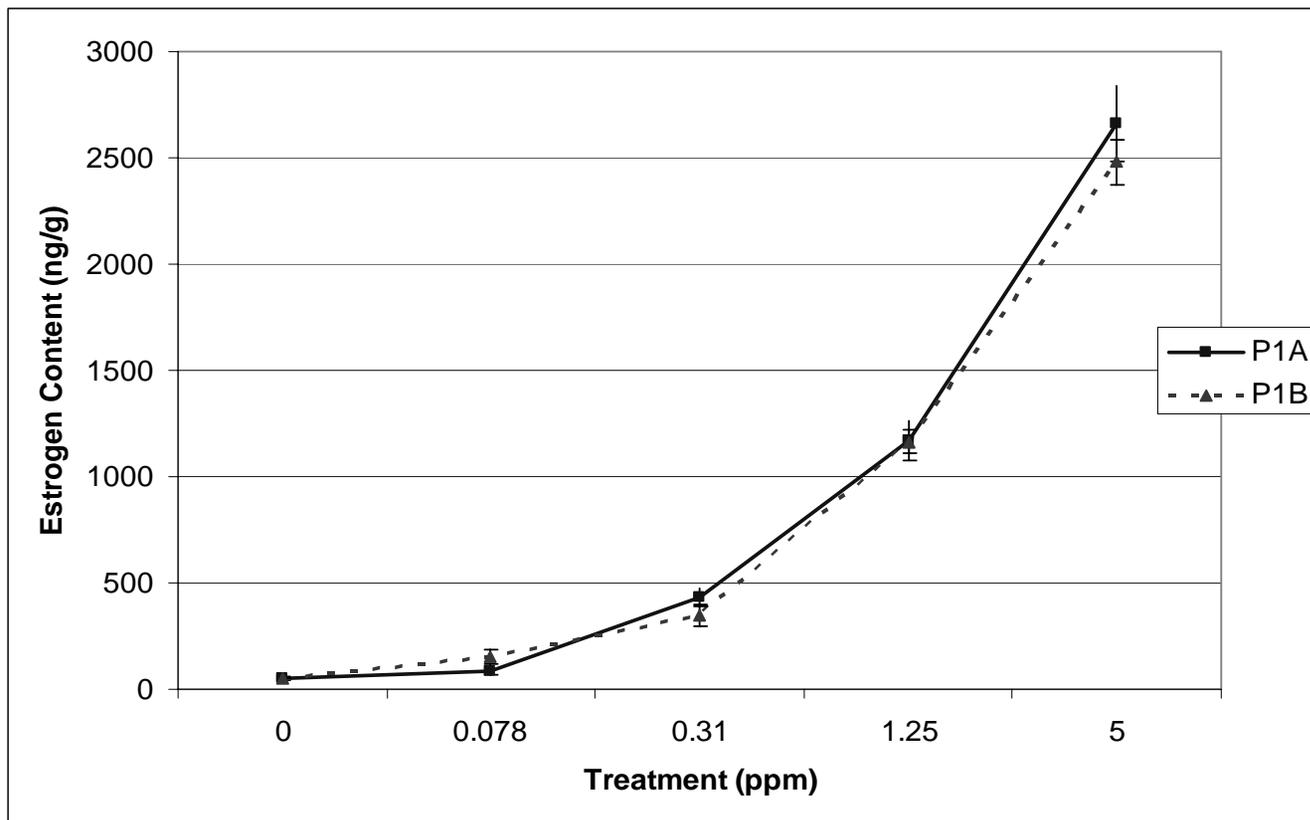
# Egg testosterone content for P1 generation



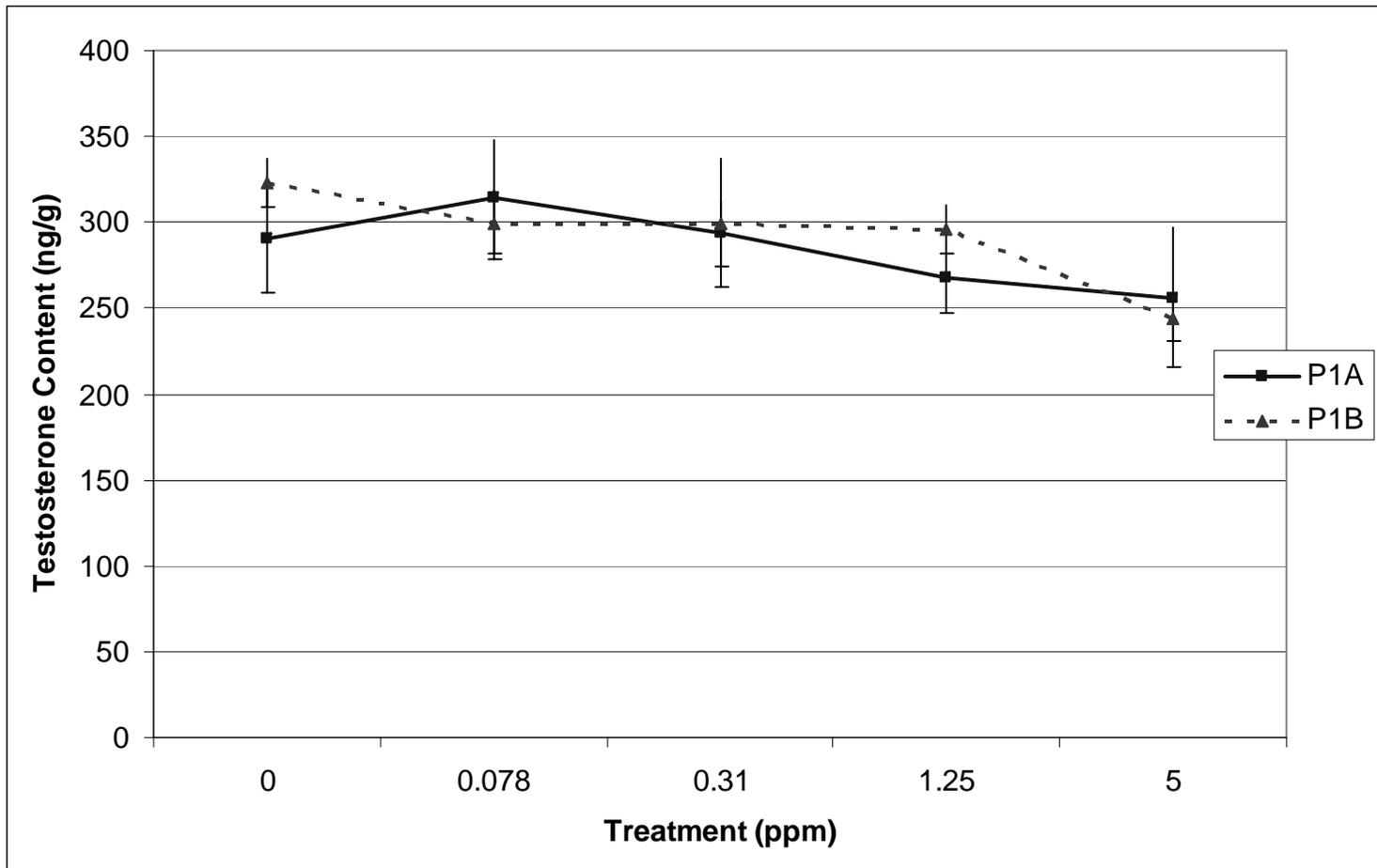
# Fecal-urate estrogen content in P1 generation females



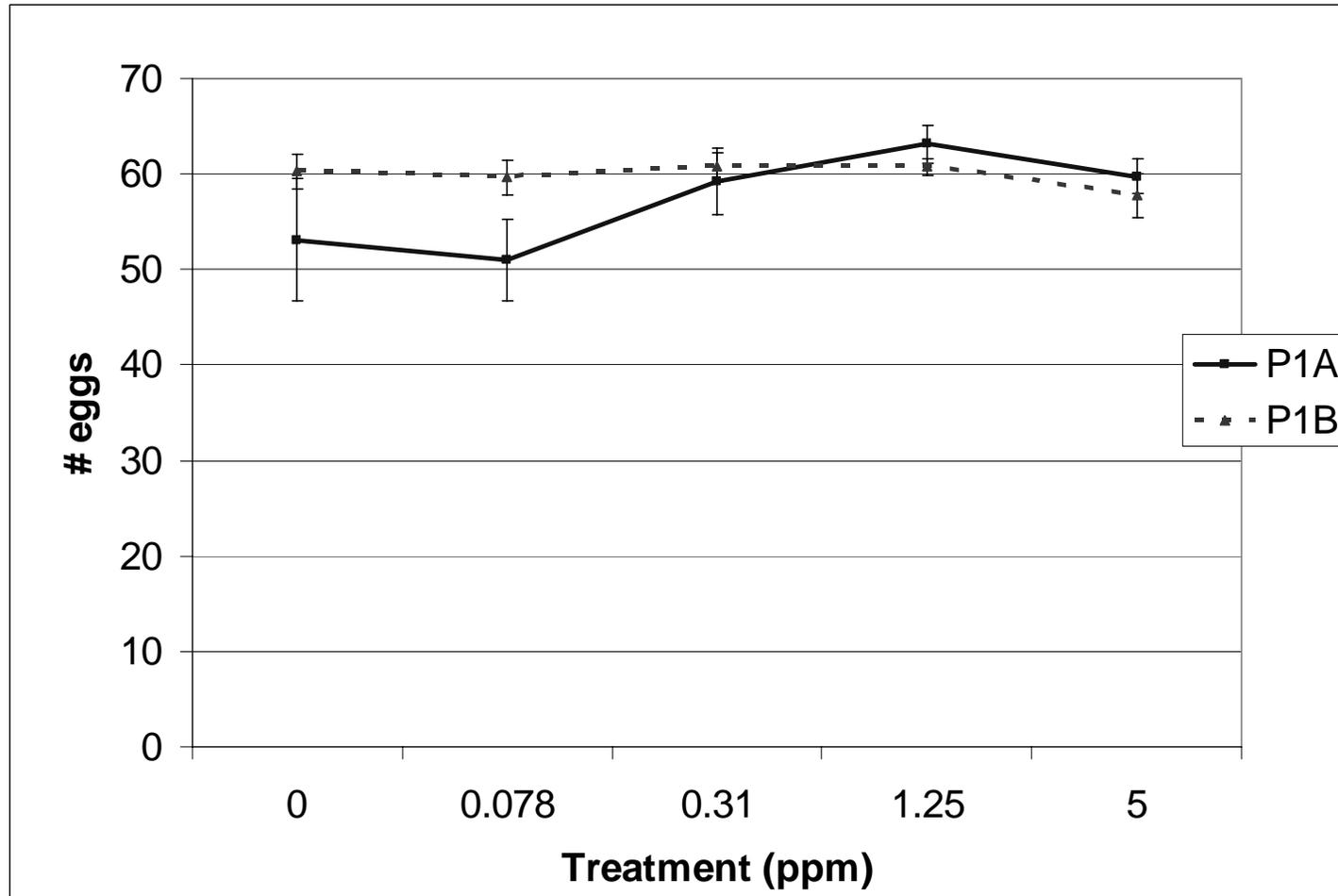
# Fecal-urate estrogen content in P1 generation males



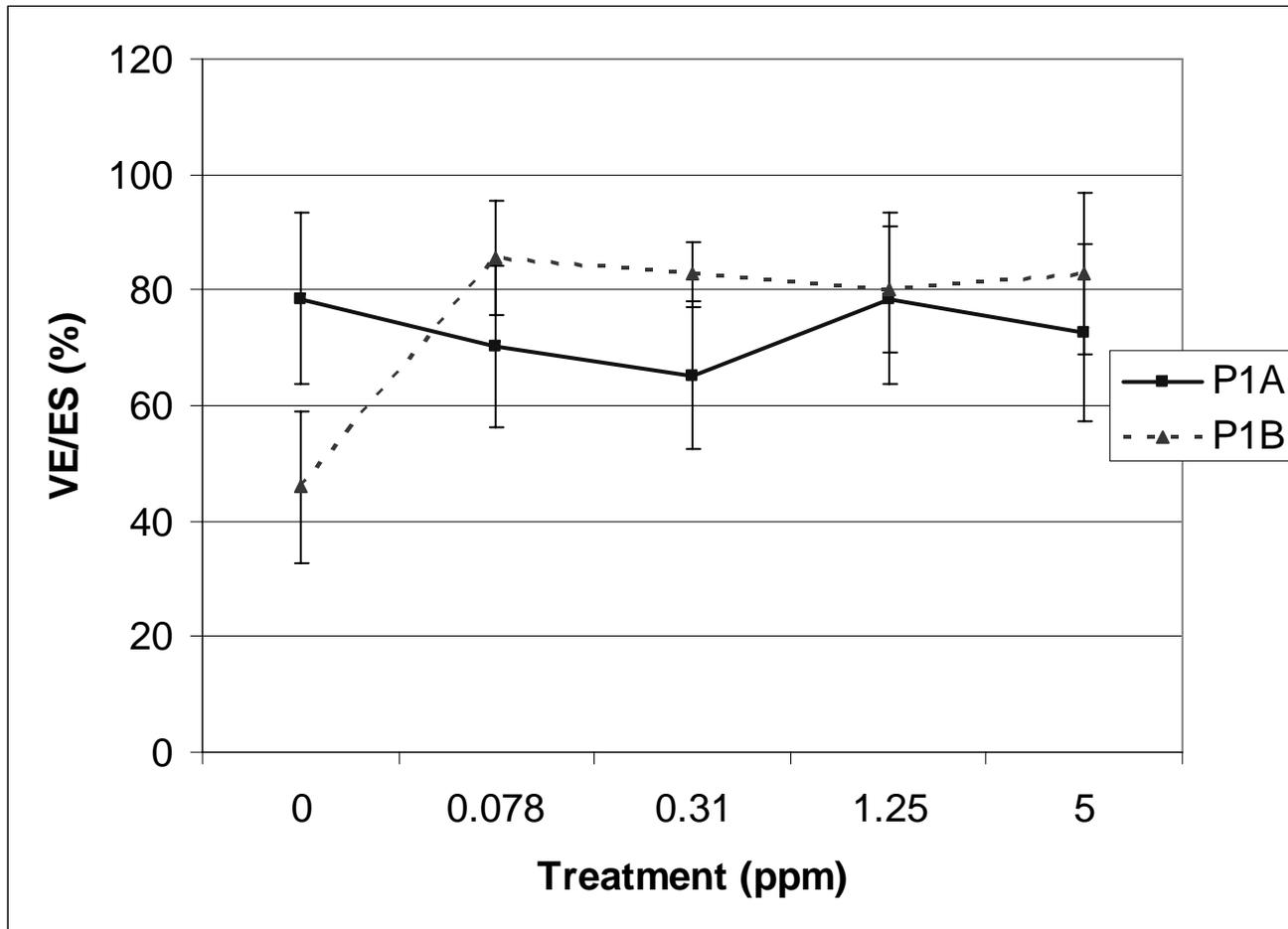
# Fecal-urate testosterone content in P1 generation males



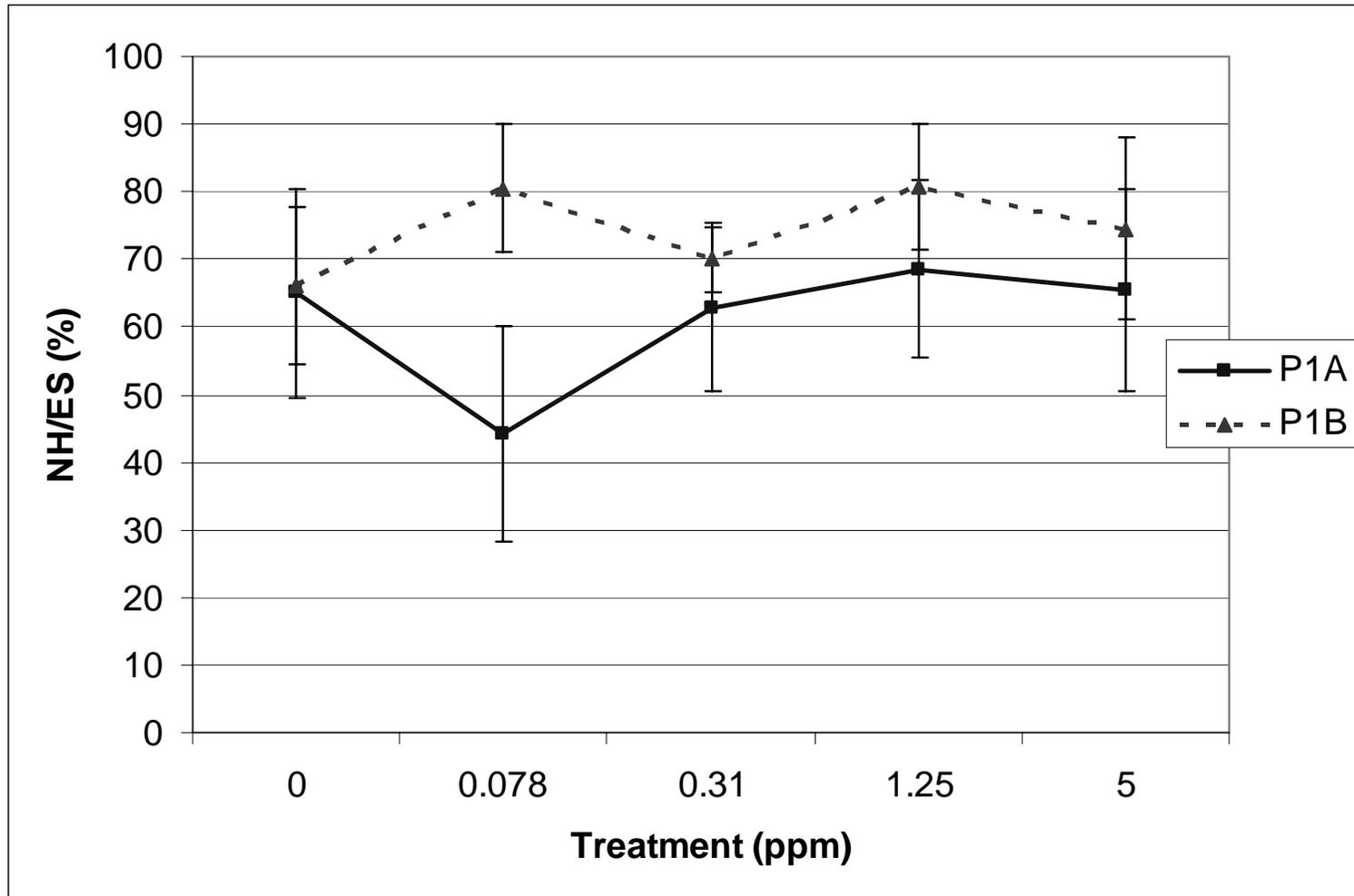
# Total eggs (mean) laid by P1 generation



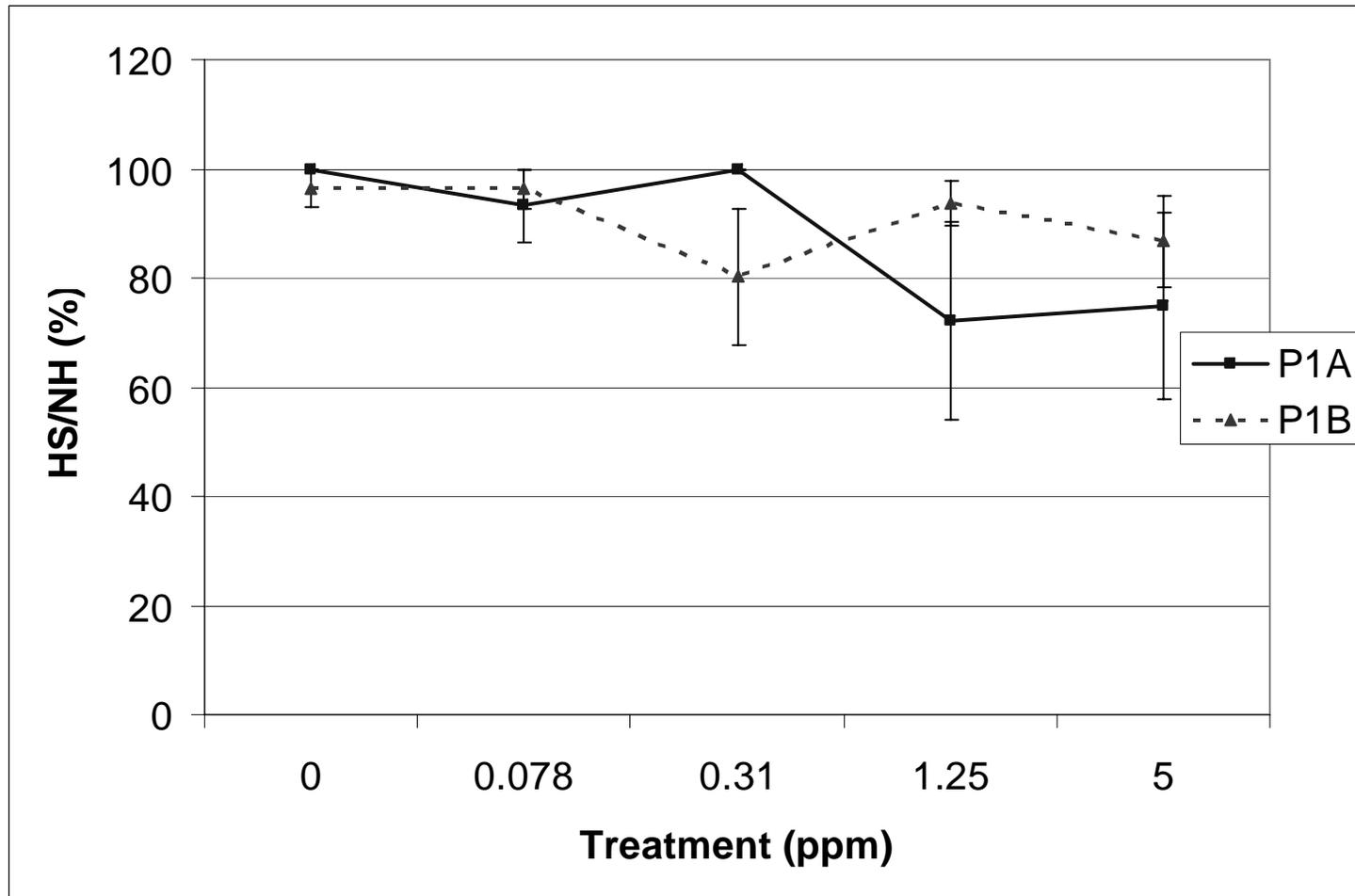
# Viability embryos (d8) out of eggs set for P1 generation



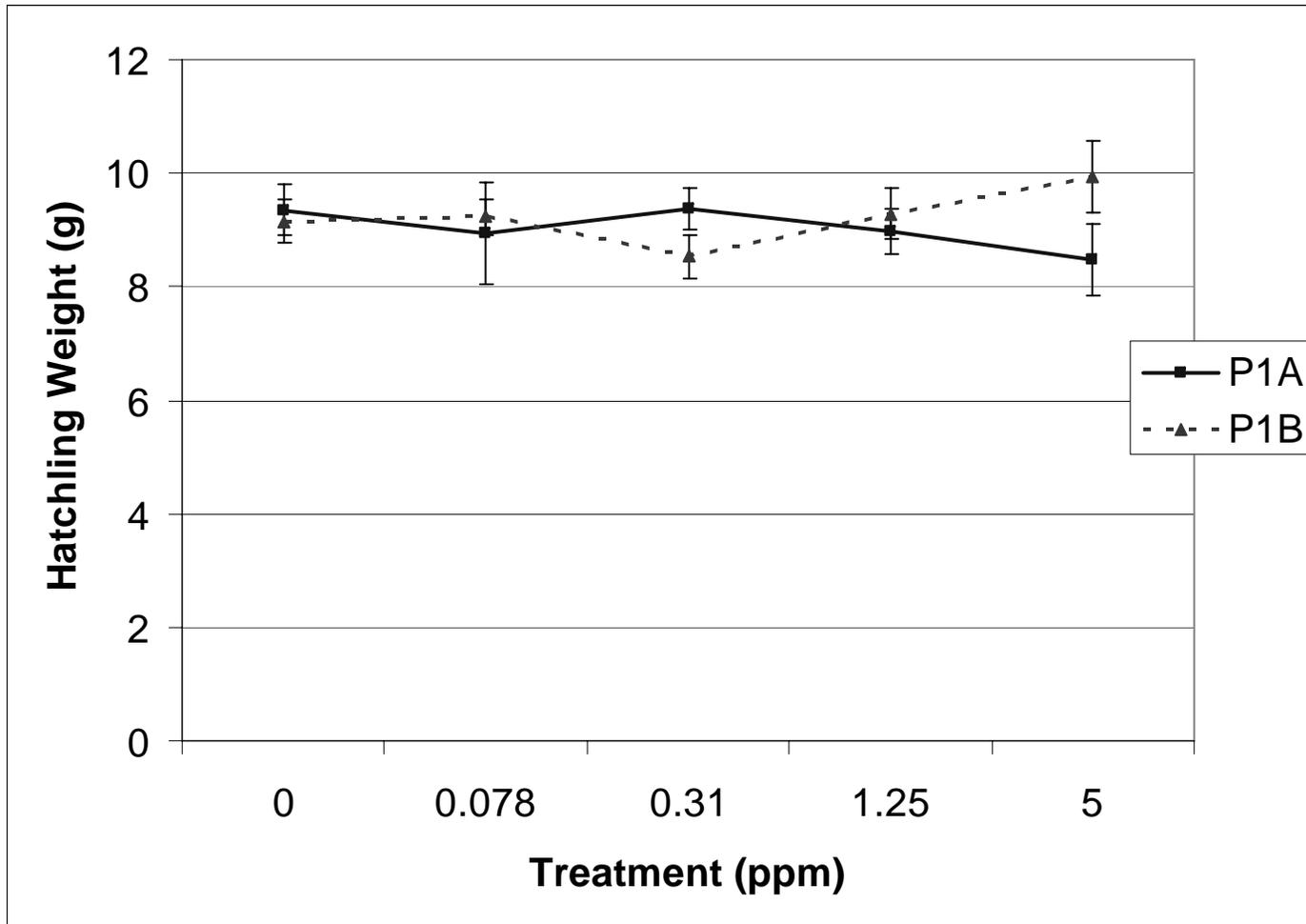
# Number hatched out of eggs set for P1 generation



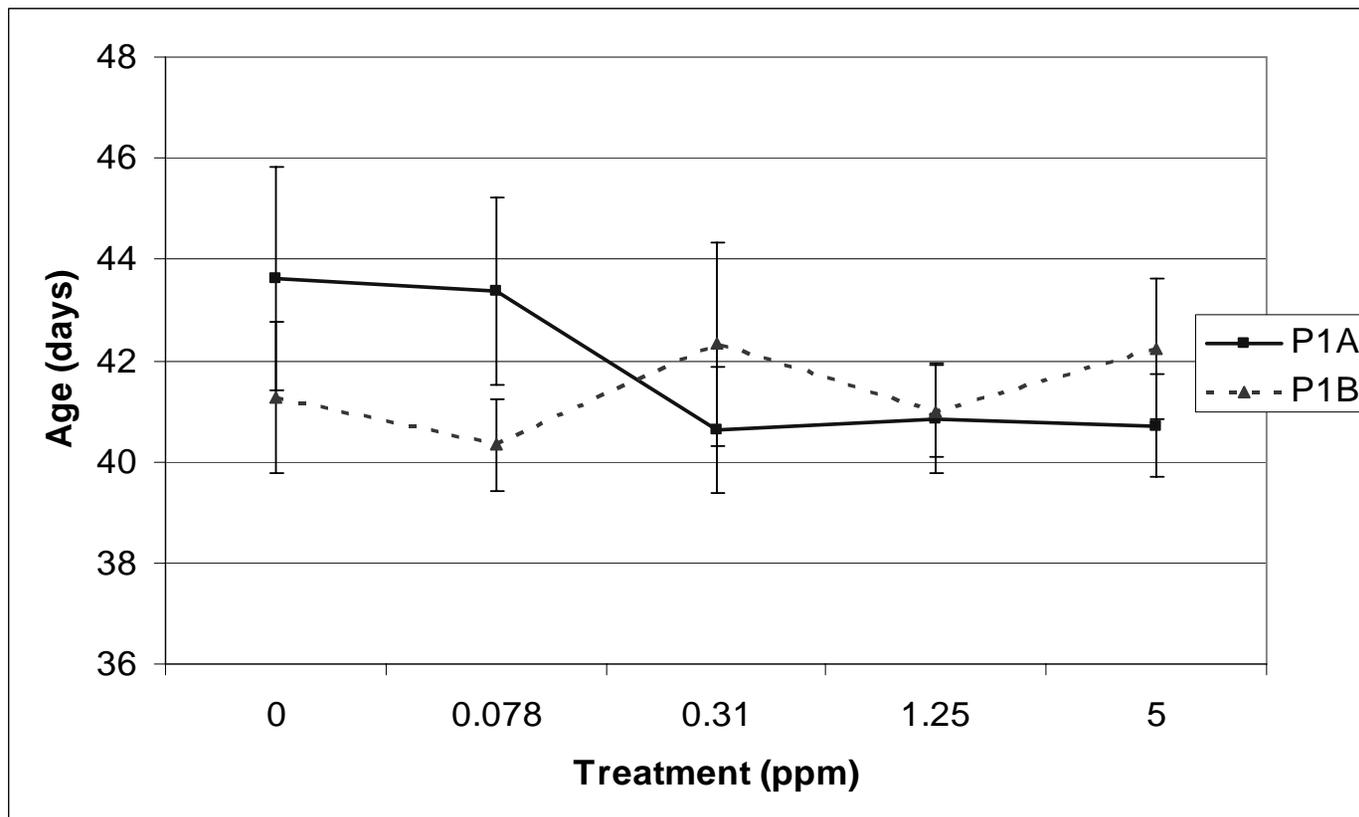
# Hatchling survival out of number hatched for P1 generation



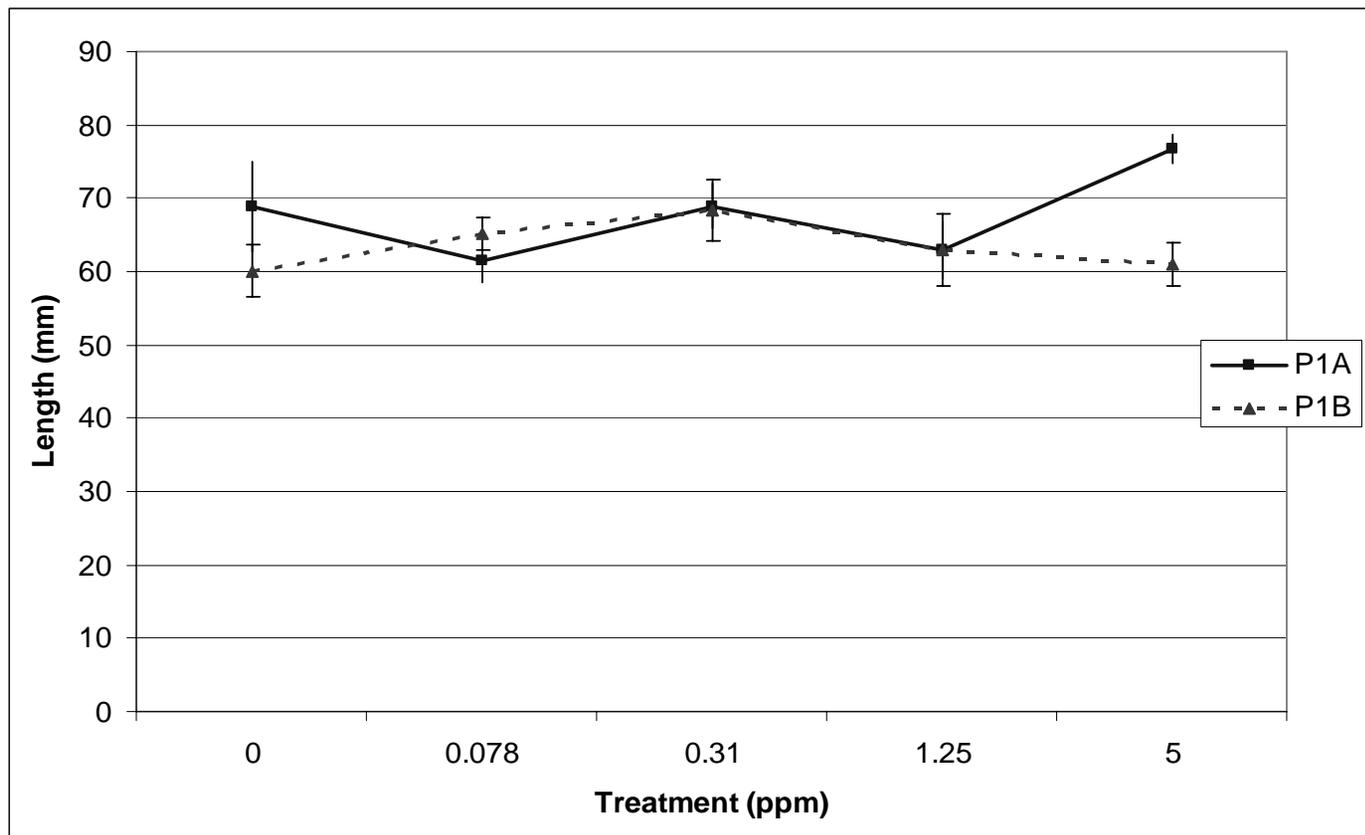
# Hatchling weight (mean) per pen for P1 generation



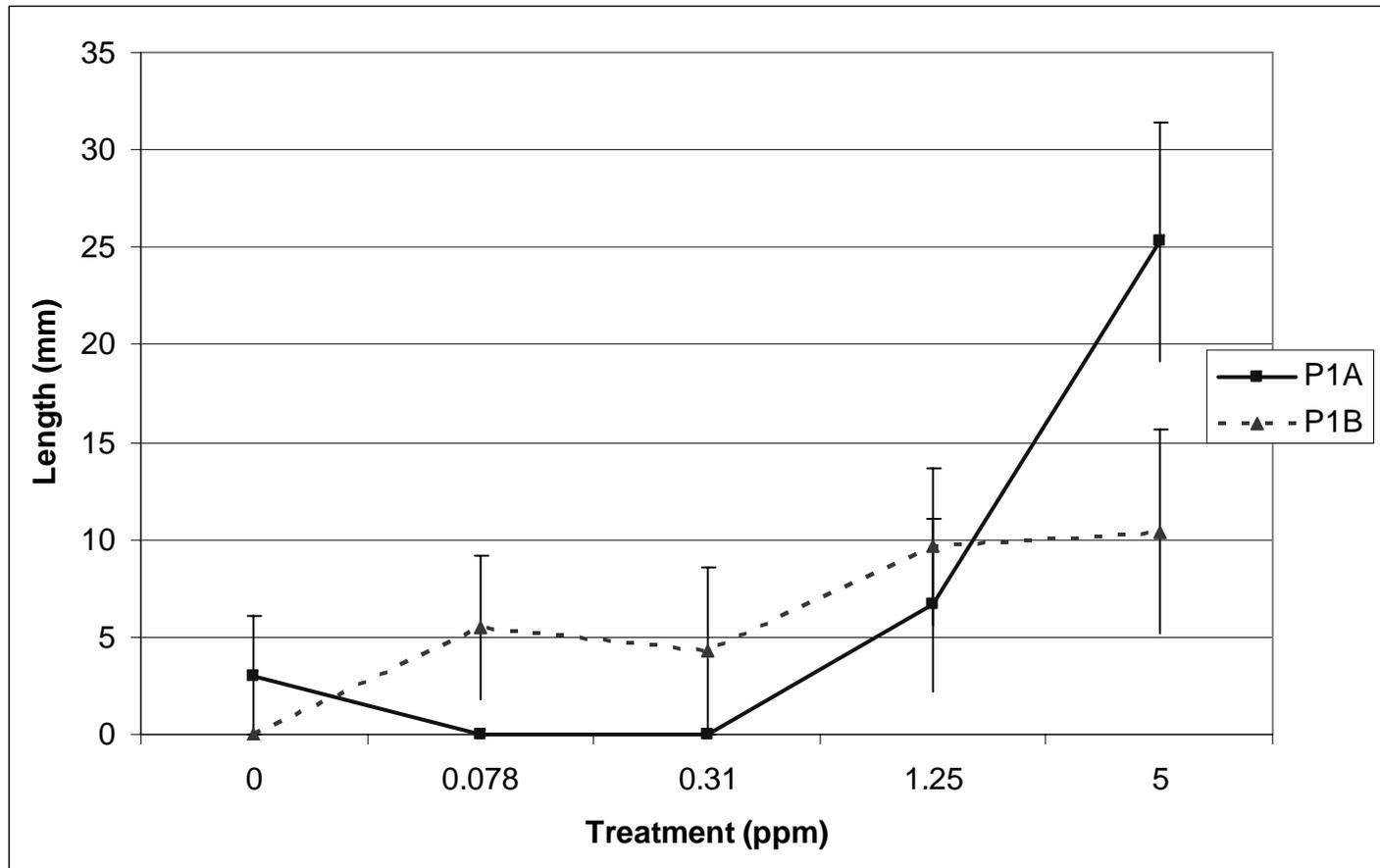
# Days to onset of egg-laying in P1 generation



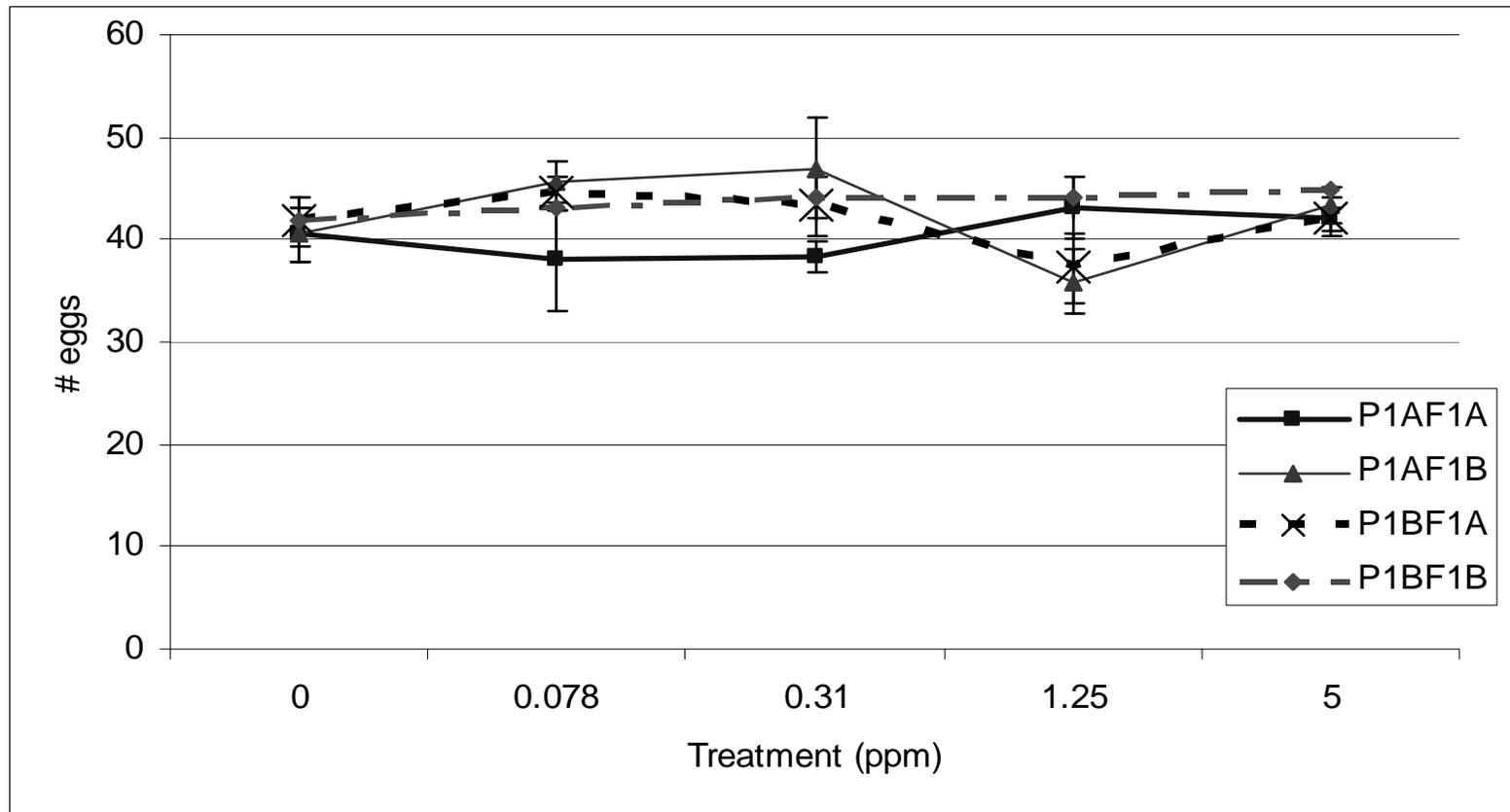
# Length of female-type plumage in P1 generation females at necropsy



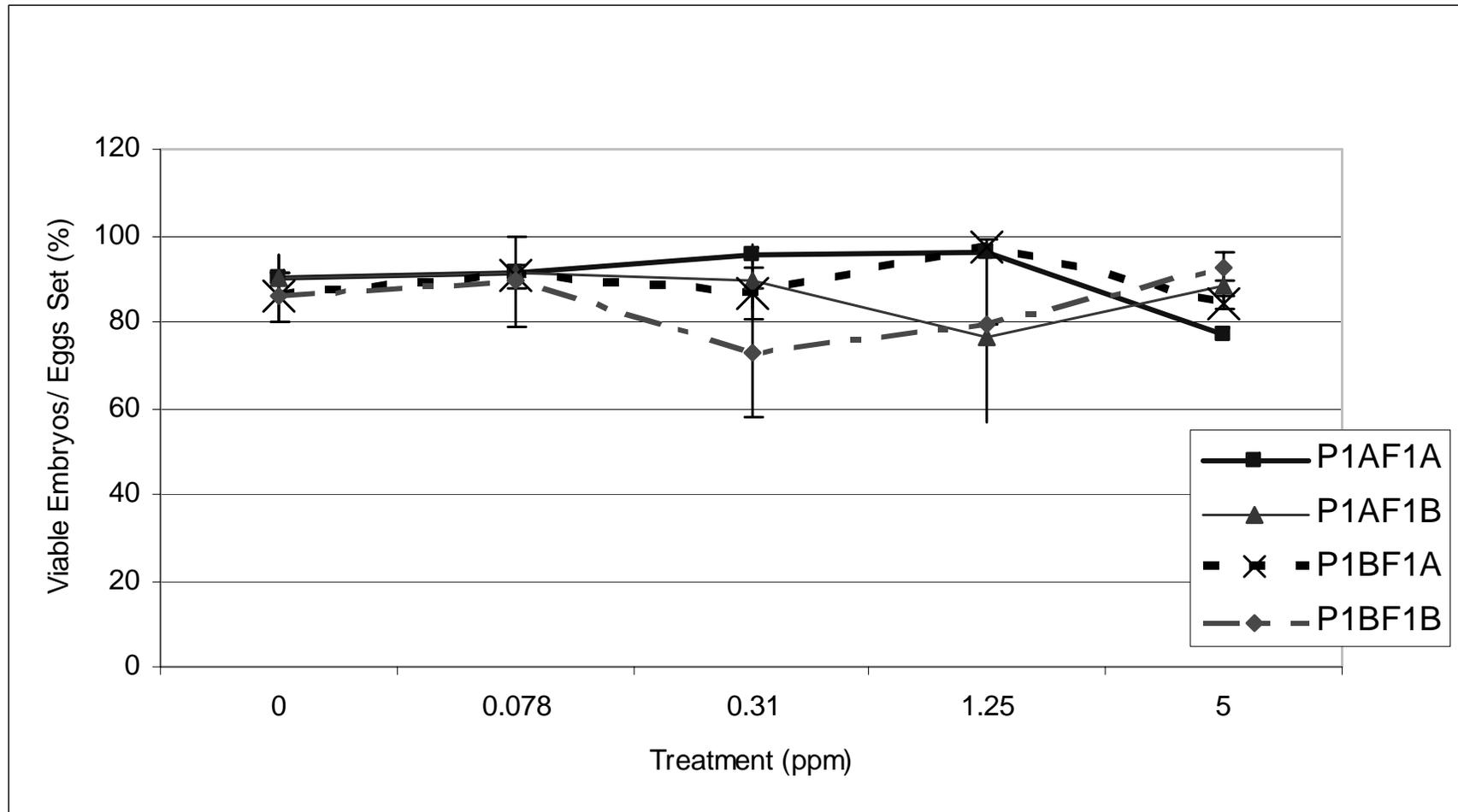
# Length of female-type plumage in P1 generation males at necropsy



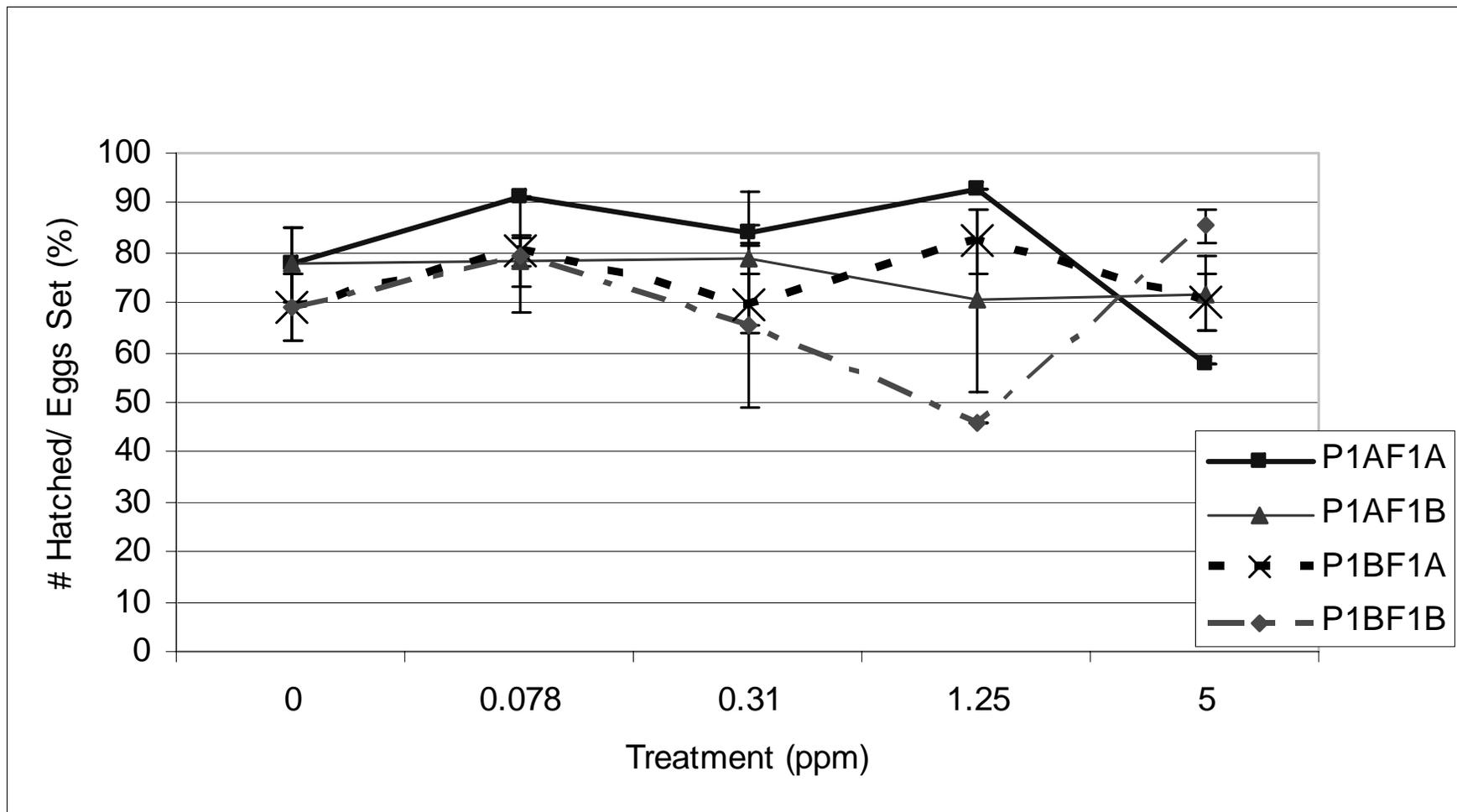
# Total eggs laid by F1 generation



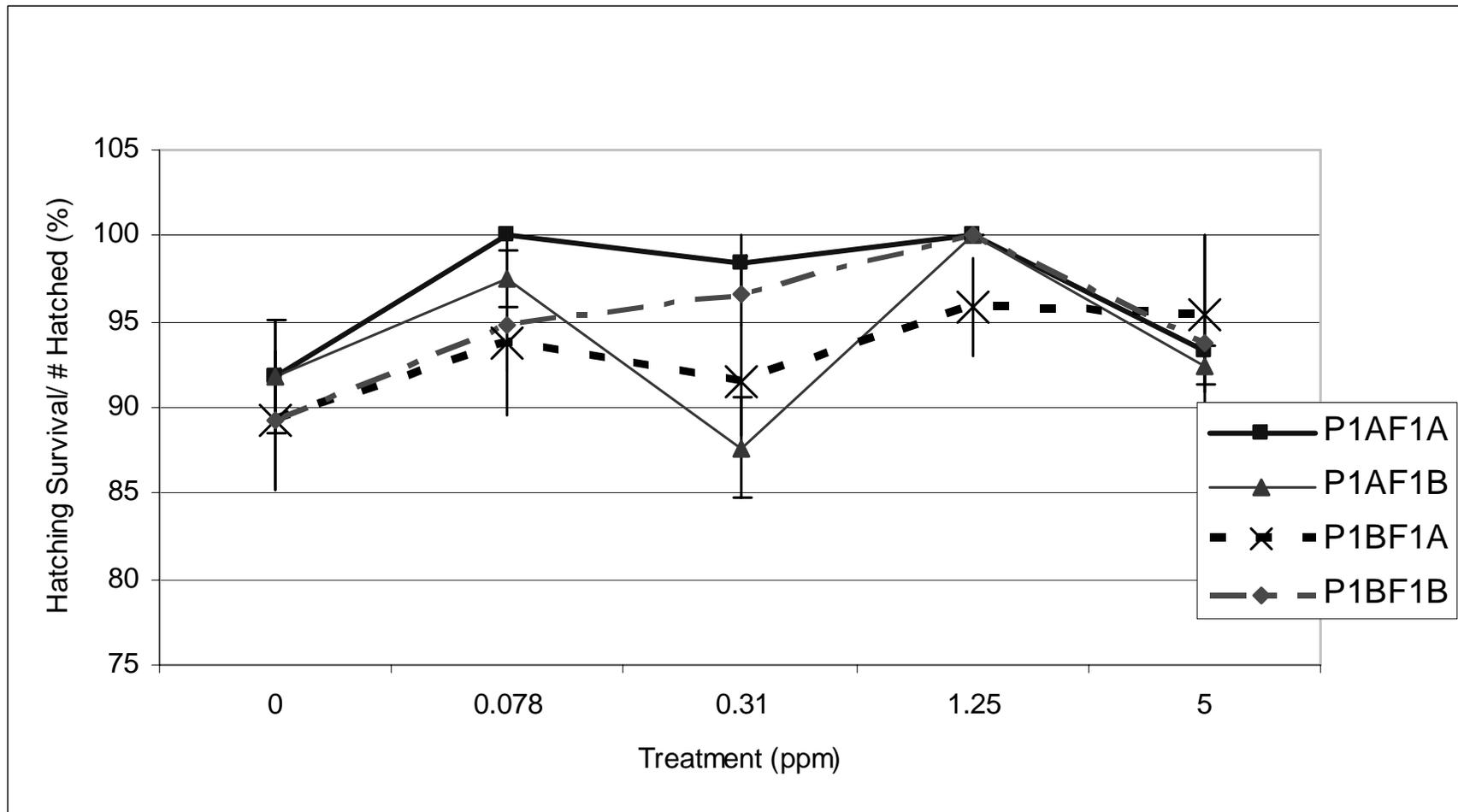
# Viable embryos (day 8) out of eggs set for F1 generation



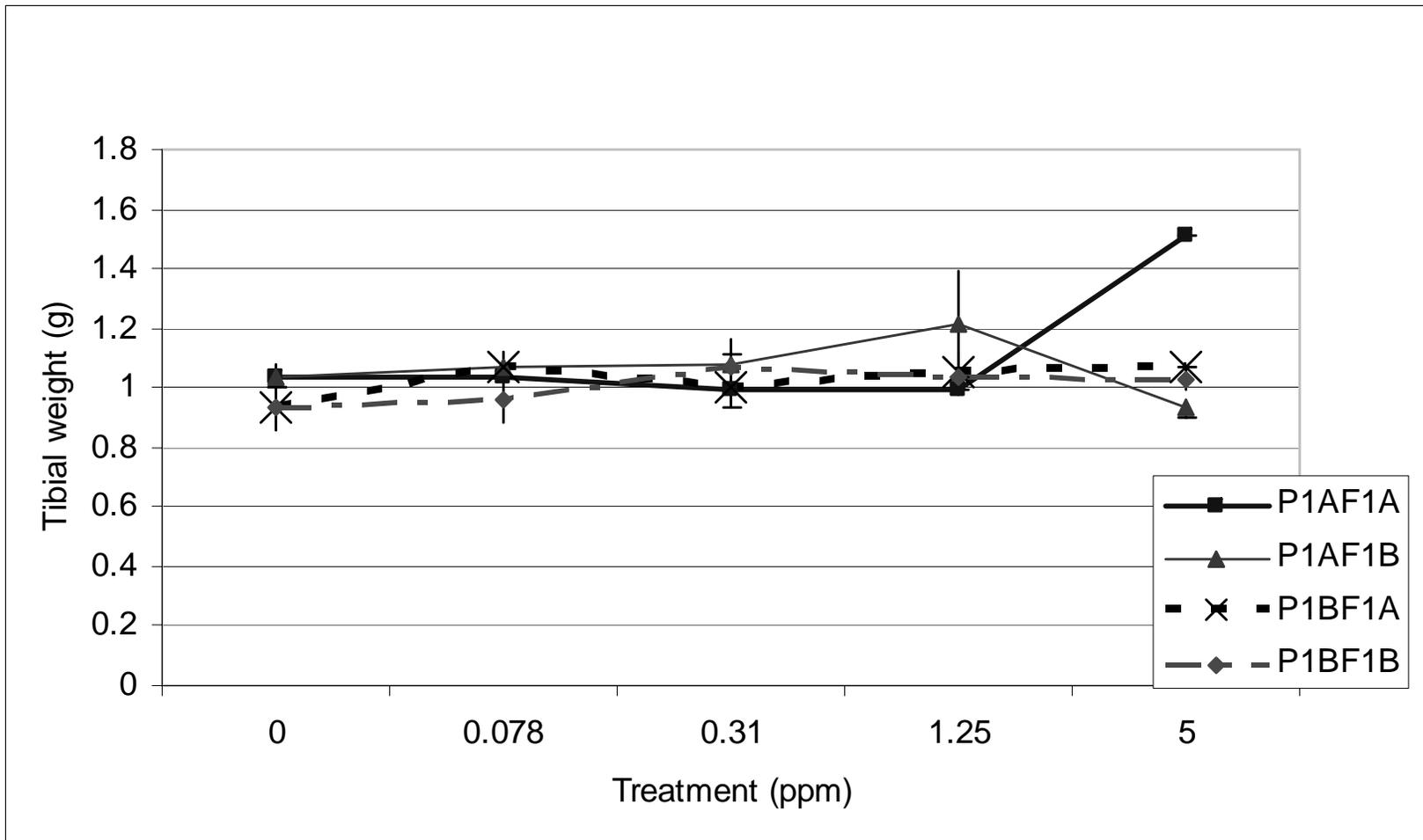
# Number hatched out of eggs set (%) for F1 generation



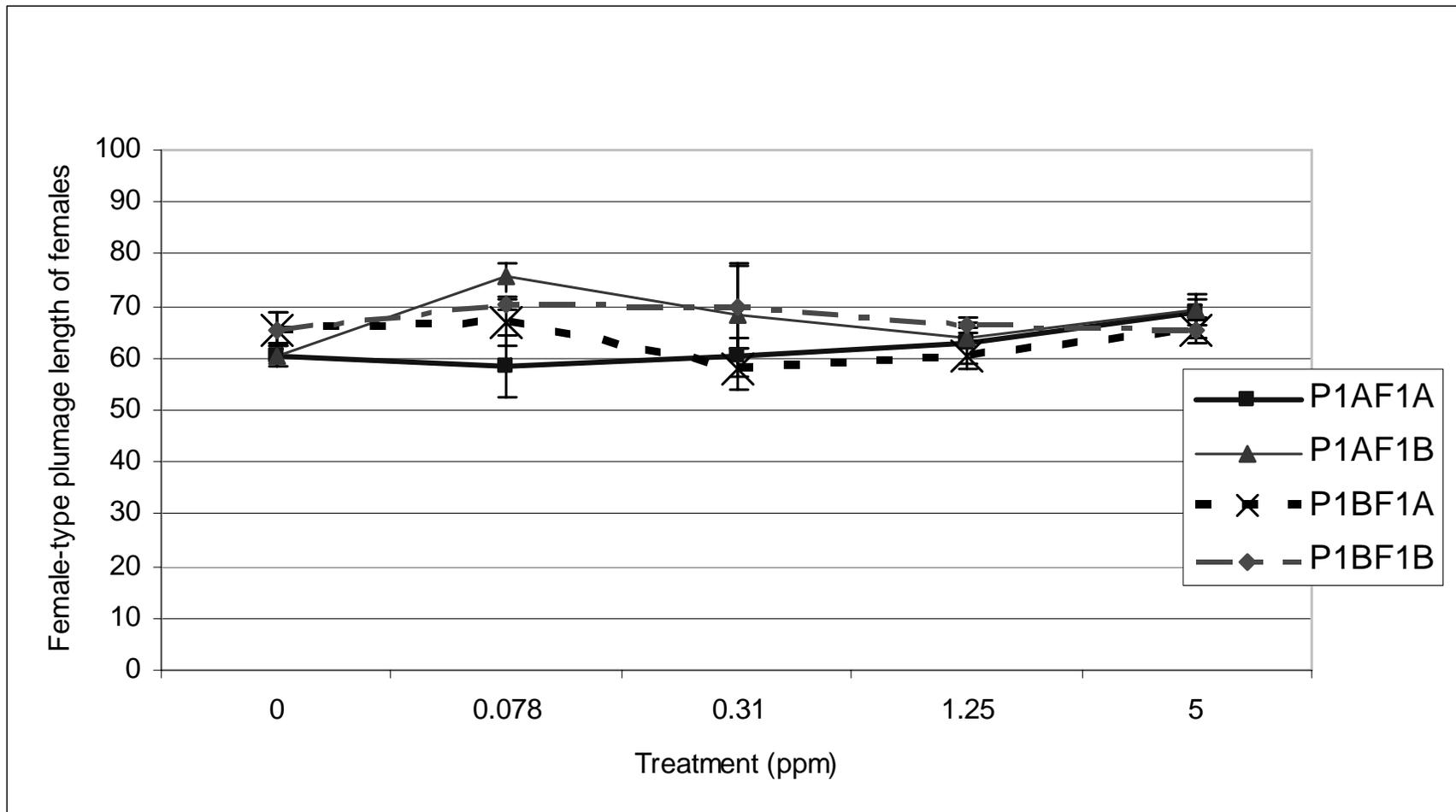
# Hatchling survival out of number hatched for F1 generation



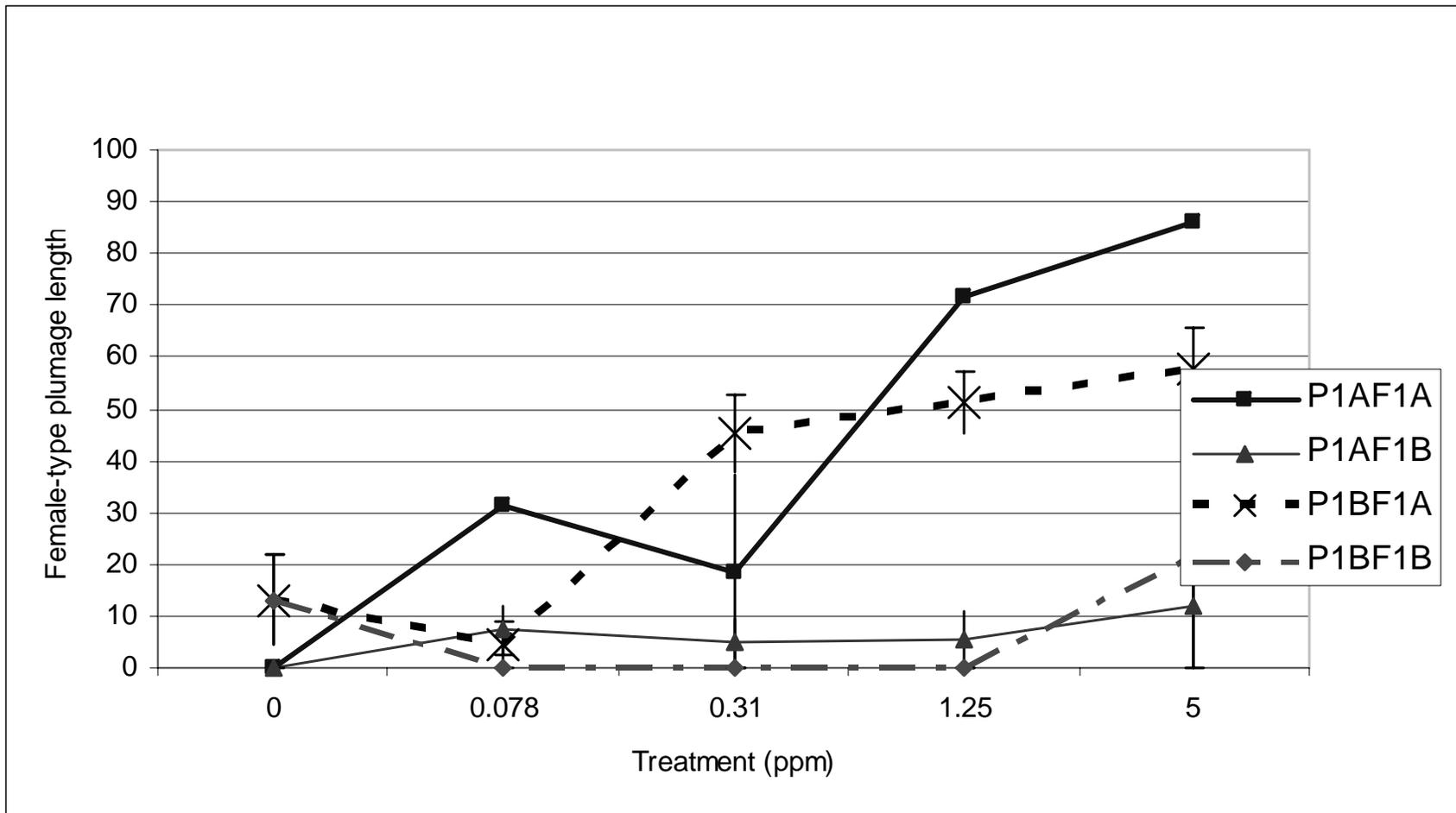
# Tibial weight of F1 generation males at necropsy



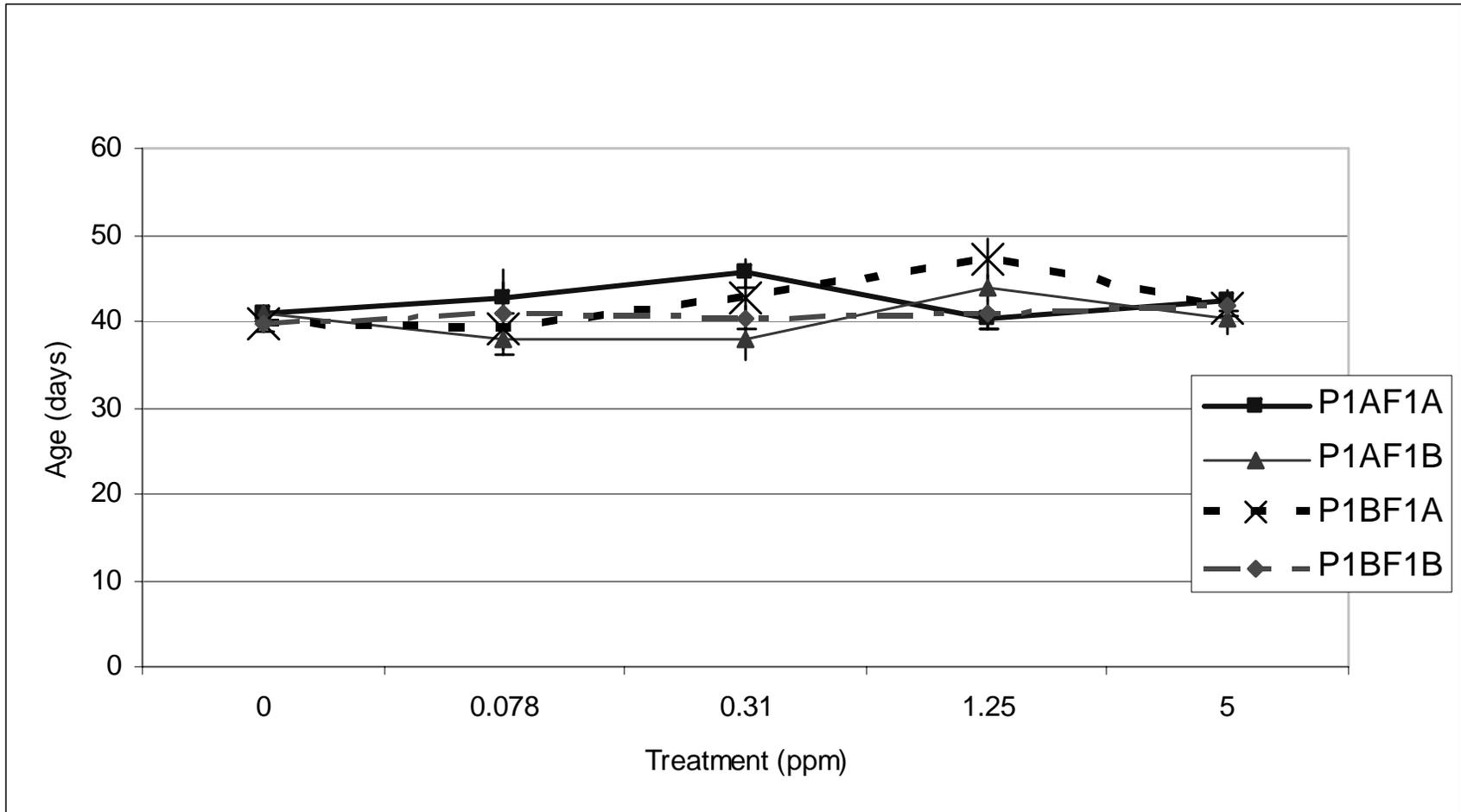
# Female-type plumage length of F1 generation females at necropsy



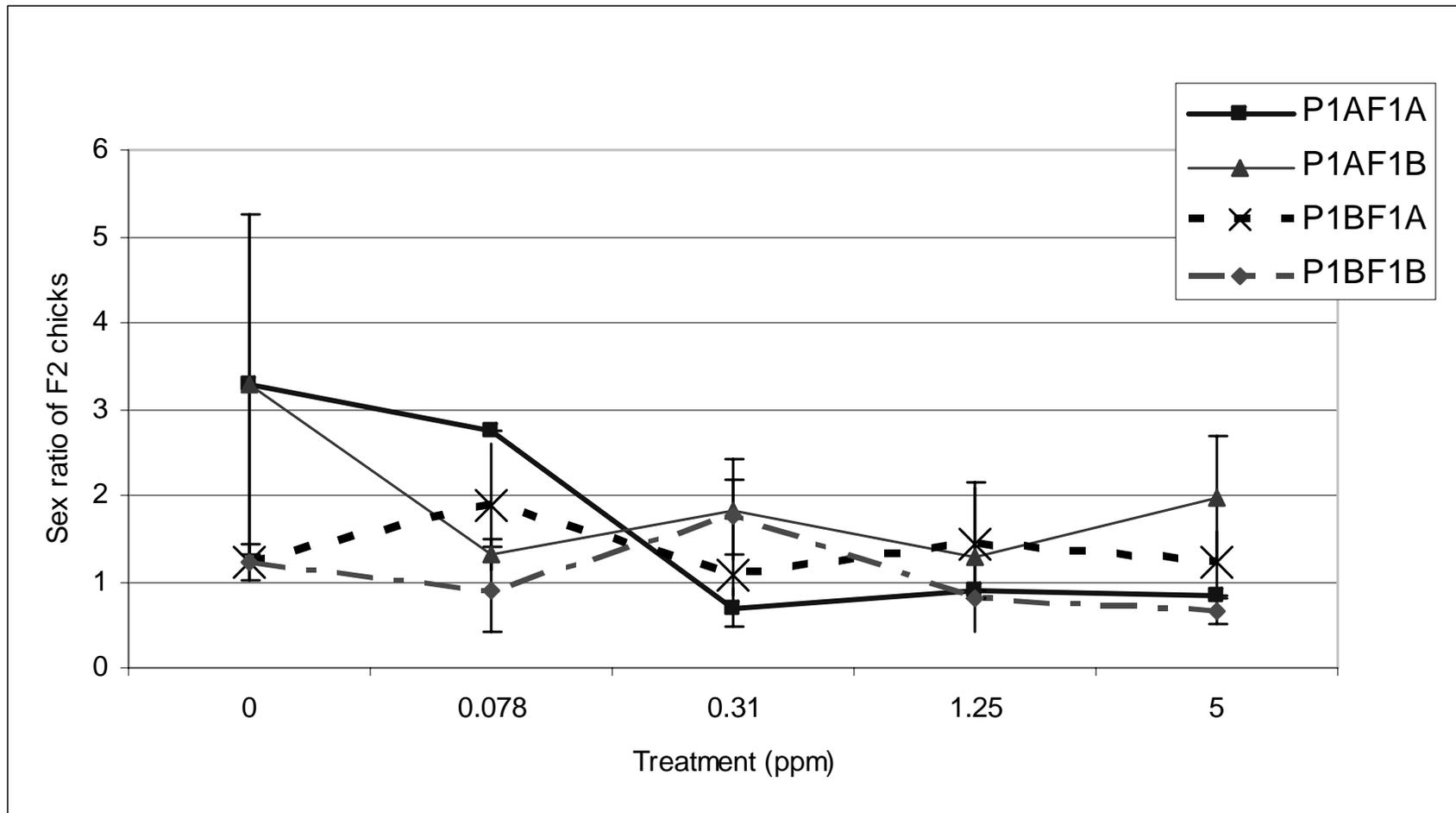
# Female-type plumage length in F1 generation males at necropsy



# Age at onset of egg-laying in F1 generation females



# Sex ratio (M:F) of F2 chicks



# Dose-related pathology findings in P1 males

- P1A males
  - Diffuse degeneration of seminiferous tubules (all groups)
  - Epididymis hypospermia (all groups)
  - Atrophy of submucosal glands of cloaca
  - Diffuse cortical and medullary hypertrophy of the adrenal gland (5 ppm)
- P1B males
  - Diffuse degeneration of seminiferous tubules (5 ppm only)

# Dose-related pathology findings in P1 females

- P1A females
  - Increased number of follicles (all groups)
  - Increased degenerating follicles (all groups)
  - Hypertrophy/hyperplasia of epithelial cells lining the oviduct
  - Diffuse cortical and medullary hypertrophy of the adrenal gland (5 ppm)
- P1B females
  - Diffuse cortical and medullary hypertrophy of the adrenal gland (all groups)

# Pathology findings in F1 males

- P1AF1A
- P1AF1B
  - Hypertrophy of submucosal glands of the cloaca (no dose-response)
  - Diffuse hypertrophy of the adrenal glands (5 ppm)
- P1BF1A
  - Increased dilatation of the submucosal glands of the cloaca
- P1BF1B
  - Diffuse degeneration of seminiferous tubules (no dose-response)
  - Epididymis hypospermia (no dose-response)
  - Increased dilatation of the submucosal glands of the cloaca
  - Diffuse hypertrophy of the adrenal glands (5 ppm)

# Pathology findings in F1 females

- P1AF1A
  - Diffuse hypertrophy of the adrenal glands (5 ppm)
- P1AF1B
  - Focal mineralization in the liver (5 ppm)
- P1BF1A
  - Diffuse hypertrophy of the adrenal glands (5 ppm)
  - Focal mineralization in the liver (5 ppm)
- P1BF1B
  - Focal mineralization in the liver (5 ppm)

# Incidence of abnormalities and injuries of P1 males

Generation and Treatment Dose	N	Presence of Injuries	Indication of Pecking	Presence of Abnormalities <sup>a</sup>	Chest feathers rubbed off	Head-neck feathers lost
P1A-0 ppm	7	1	0	1	2	0
P1A-0.078 ppm	8	0	0	2	3	2
P1A-0.31 ppm	7	3	2	2	4	1
P1A-1.25 ppm	8	1	0	1	1	3
P1A-5 ppm	8	3	2	0	2	3
P1B-0 ppm	6	2	1	0	2	4
P1B-0.078 ppm	8	2	2	0	4	0
P1B-0.31 ppm	8	2	2	0	2	3
P1B-1.25 ppm	9	1	1	0	2	2
P1B-5 ppm	8	1	0	0	3	0

<sup>a</sup> Abnormalities denote various organ lesions

# Incidence of abnormalities and injuries of P1 females

Generation and Treatment Dose	N	Presence of Right Ovary	Presence of Right Oviduct	Presence of Injuries	Indication of Pecking	Presence of Abnormalities <sup>a</sup>	Chest feathers rubbed off	Head-neck feathers lost
P1A-0 ppm	8	0	1	2	1	5	0	5
P1A-0.078 ppm	8	1	1	4	2	3	1	6
P1A-0.31 ppm	7	0	0	0	0	2 <sup>b</sup>	2	3
P1A-1.25 ppm	7	0	1	1	1	2 <sup>b</sup>	0	5
P1A-5 ppm	7	0	1	3	3	3	1	5
P1B-0 ppm	7	0	2	0	0	3	0	6
P1B-0.078 ppm	8	0	5	2	2	1	1	6
P1B-0.31 ppm	7	0	5	0	0	1 <sup>b</sup>	1	4
P1B-1.25 ppm	8	0	2	1	0	1	1	3
P1B-5 ppm	8	0	3	0	0	2	2	5

<sup>a</sup> Abnormalities include organ lesions.

<sup>b</sup> One incident of egg binding.

# Summary

- Majority of endpoints not affected
- Length of feminized plumage in males most apparent endpoint affected
- 14-day survival of P1A chicks:  
    NOEC=1.25ppm LOEC=5ppm
- P1AF1A group most affected

# Issues/Concerns

- Animal usage
- Value added of 2 gen v. 1 gen
- Japanese quail sensitivity
- Lack of avian assay in Tier 1
- Time delay in validation (& costs)
- Linkage with existing avian testing framework

# Recommended Test Guidelines

- Based on P1AF1A results (pre-egg-laying treatment and treated chicks)
- Initiate with sufficient pairs to ensure a minimum of eight (8) pairs per in the P generation control group at the end of treatment
- Set a minimum of 6 eggs per pair (40 per level) from 6<sup>th</sup>-7<sup>th</sup> weeks of exposure
- A minimum of eight pairs per test level are established with the F1 survivors
- The test is terminated with 14-day survival observation of the 6<sup>th</sup> week offspring (F2)

# Next Steps

- Initiate a demonstration study with revised protocol (Suggested compound - endosulfan)  
2006-2007
- Repeat in second laboratory to confirm transferability  
2007-2008
- Perform an inter-laboratory trial (3 lab minimum)  
2009

## Avian Dosing Study Questions for the EDMVAC

1. Please take note of the Avian Dosing Study Report and make comments on:
  - the appropriateness of the statistical analyses performed, and
  - the recommendations for the preferred dosing regimen in a Japanese quail two- generation toxicity test method.
2. Please take note of the draft OECD proposal for a new test guideline Avian Two Generation Toxicity Test in the Japanese Quail and provide comments to consider in further revision.