CHARITÉ CAMPUS BENJAMIN FRANKLIN

Integration of external and skeletal anomalies in Japanese quail into the new terminology

WHO - Collaborating Center for Developmental Toxicology

Konstanze Grote
Charité University Medical School Berlin
Campus Benjamin Franklin
Institute of Clinical Pharmacology und Toxicology

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Introduction

- Convincing evidence from field and lab that morphological development of bird offspring *in ovo* may be altered by exposure of either the hen or the eggs to environmental contaminants (*e.g.,* Hoffman and Albers, 1984; Albers *et al.*, 2001; Fernie *et al.*, 2003; Kertész *et al.*, 2006).

Introduction

- In contrast to mammalian toxicology in which teratogenicity and developmental toxicity of drugs or pesticides are mandatory endpoints in regulatory studies, comparable investigations in avian species are legally not required (OECD, 1984; OECD, 2000).

Introduction

- We report findings from skeletal examination of control chicks sacrificed immediately post hatch in several one-generation reproduction studies in Japanese quail (*Coturnix coturnix japonica*).
- Studies were part of an extensive research project that was carried out in cooperation with the BfR between 2001 and 2004 (Funding: German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety).

Introduction

- Main objective of studies was to check whether additional toxicological endpoints, such as spermatid count, hormone concentrations or sex ratio in chicks, might be suitable to improve detection and characterisation of adverse effects of xenobiotics on fertility and reproductive success in birds.
- Not designed for evaluation of embryotoxicity !

Methods

- Studies were performed according to a proposal for a new guideline for an avian reproduction test in Japanese quail (OECD, 2000).
- 13 to 20 week old adult birds were administered the test substances via their diet or **untreated feed** for six weeks.
- 16 to 18 breeding pairs per dose were employed.

Methods

- Daily collection of eggs and storage at 16 ± 1°C and 60 – 70% humidity for a maximum of seven days.
- Incubation for 2 weeks at 37.8 ± 0.1°C and 60 ± 5% relative humidity and automatic turnover several times a day.
- Three or four days before expected hatch exposure to higher humidity (80 ± 5%).
- Hatched chicks were assessed for viability and external malformations.
- Chicks hatched from eggs layed during weeks 1, 3, and 5 were killed, necropsied and prepared for subsequent skeletal examination.

Methods

- Method for skeleton preparation from Chahoud et al.(1988) and Faqi et al.(1997) modified for 13- to16-day quail embryos and newly hatched chicks (Stoll, 2002).
- Findings were described according to the internationally harmonised nomenclature for common laboratory mammals (Wise et al.,1997; Makris et al., 2009) unless otherwise stated.

Results

- A control database of 793 one-day old chicks that hatched from eggs layed by untreated hen in several one-generation studies was compiled.
- Table summarises the skeletal anomalies that were recorded in the historical control animals with a frequency at or above 1%.

Results

Skeletal region	Finding	Chicks affected (relative incidence)
Hindlimb	Irregular position of toes (predominantly toe 3 and/or 4)	42 (5.3 %)
Pelvis	Incomplete ossification (mostly ilium affected) *	88 (11.1 %)
Thorax	Furcula bent or otherwise irregularly shaped	8 (1.0 %)
Vertebral column, lumbar and/or sacral vertebrae	Incomplete ossification*	14 (1.8 %)
Vertebral column, caudal vertebrae	Incomplete ossification*	12 (1.5 %)

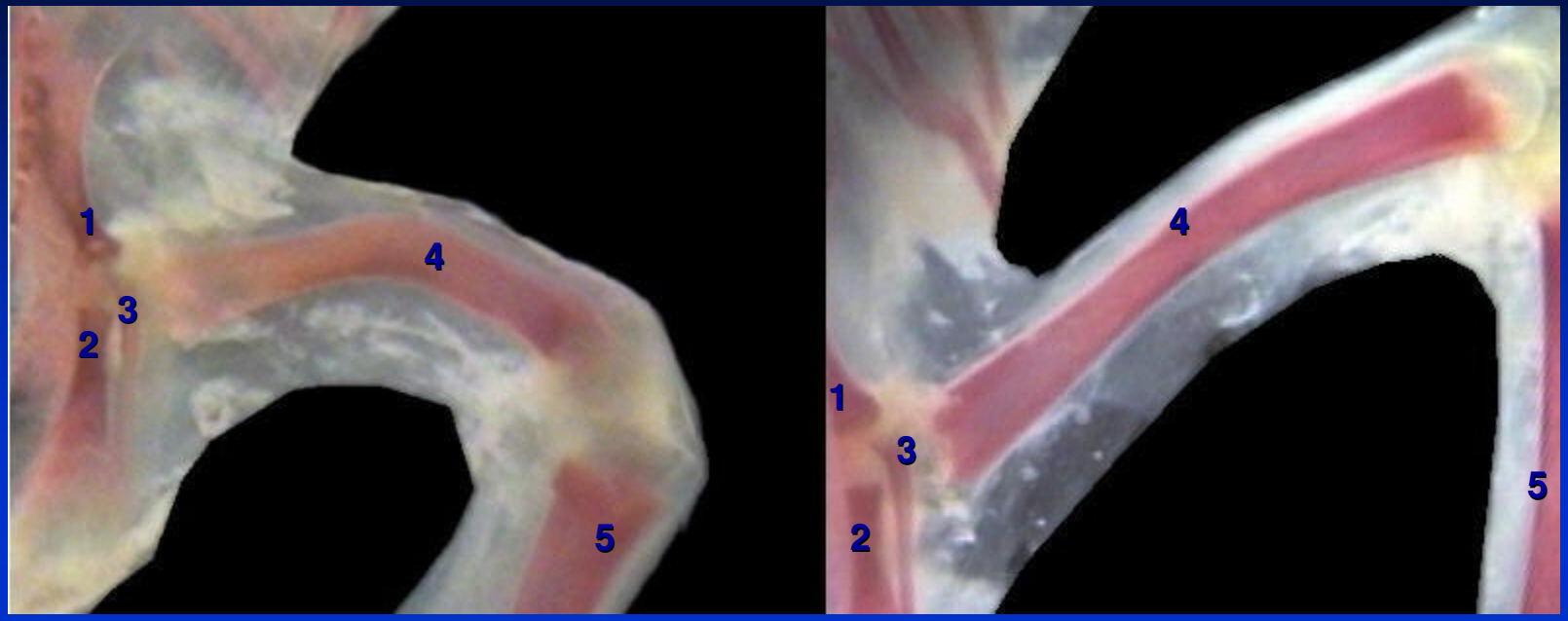
(Niemann et al., in preparation)

Results

Hindlimb skeletal findings

Femur	bent
Femur	shorter
Femur prox.	bent
Femur prox.	irreg.spong.
Tibia	bent
Tibia dist.	irreg.spong.
Tibia prox.	bent
Tibia prox.	irreg.spong.
Toe1	irreg.pos.
Toe2	irreg.pos.
Toe3	irreg.pos.
Toe4	irreg.pos.
TPh dis 3	irreg.pos.

*The term “incomplete ossification” also comprises the findings “irregular” or “poor ossification” and “irregular spongiosa” that were used in some of the earlier studies and are not in line with current terminology



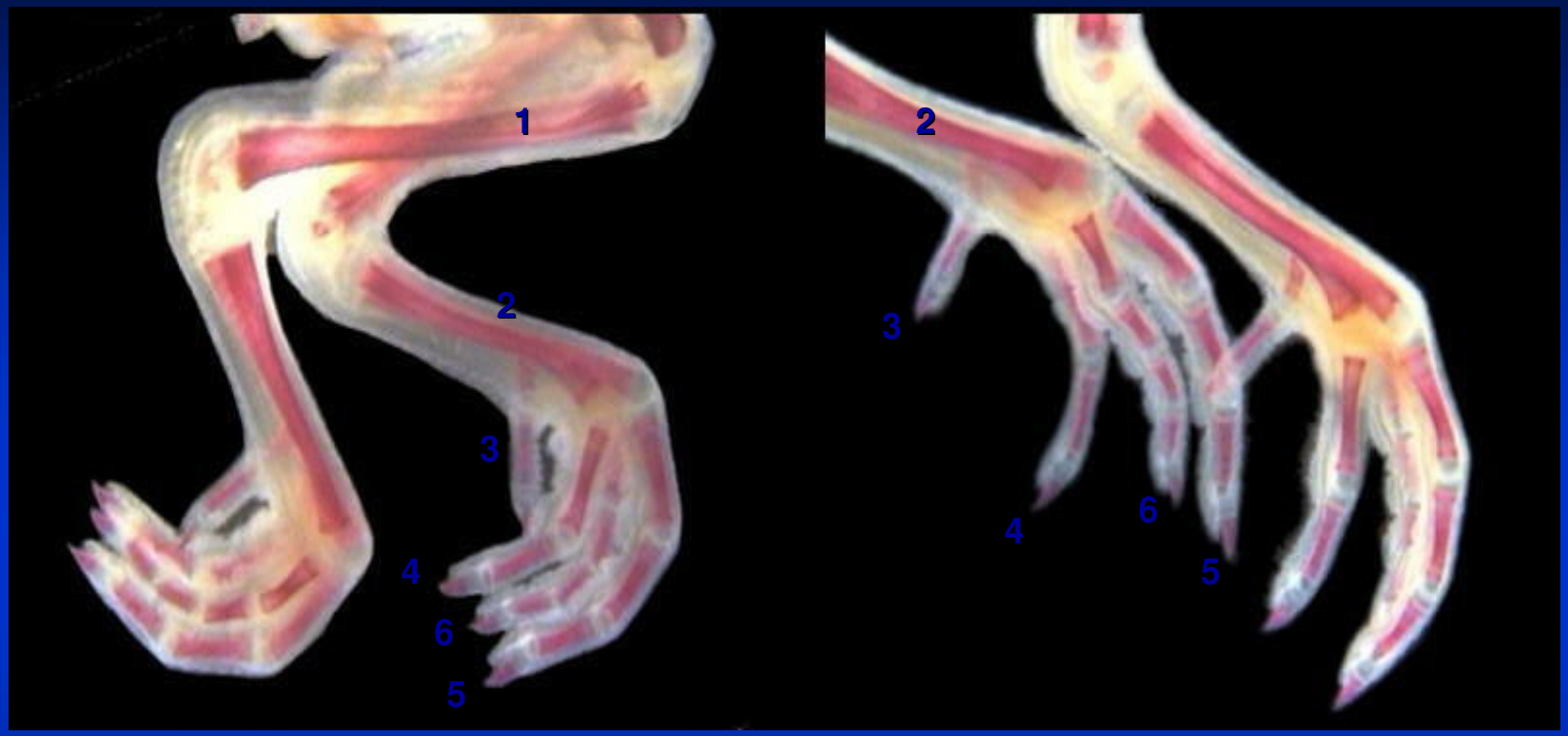
Femur bent **Control**

Femur 1: Os ilium. 2: Os ischii. 3: Os pubis. 4: Os femoris . 5: Tibiotarsus

Results

Hindlimb external findings

Hindpaw	Hyperextension
Hindpaw	Hyperflexion
Leg	Hyperextension
Leg	splayed
Toes	Hyperextension
Toes	Hyperflexion
Toes	splayed



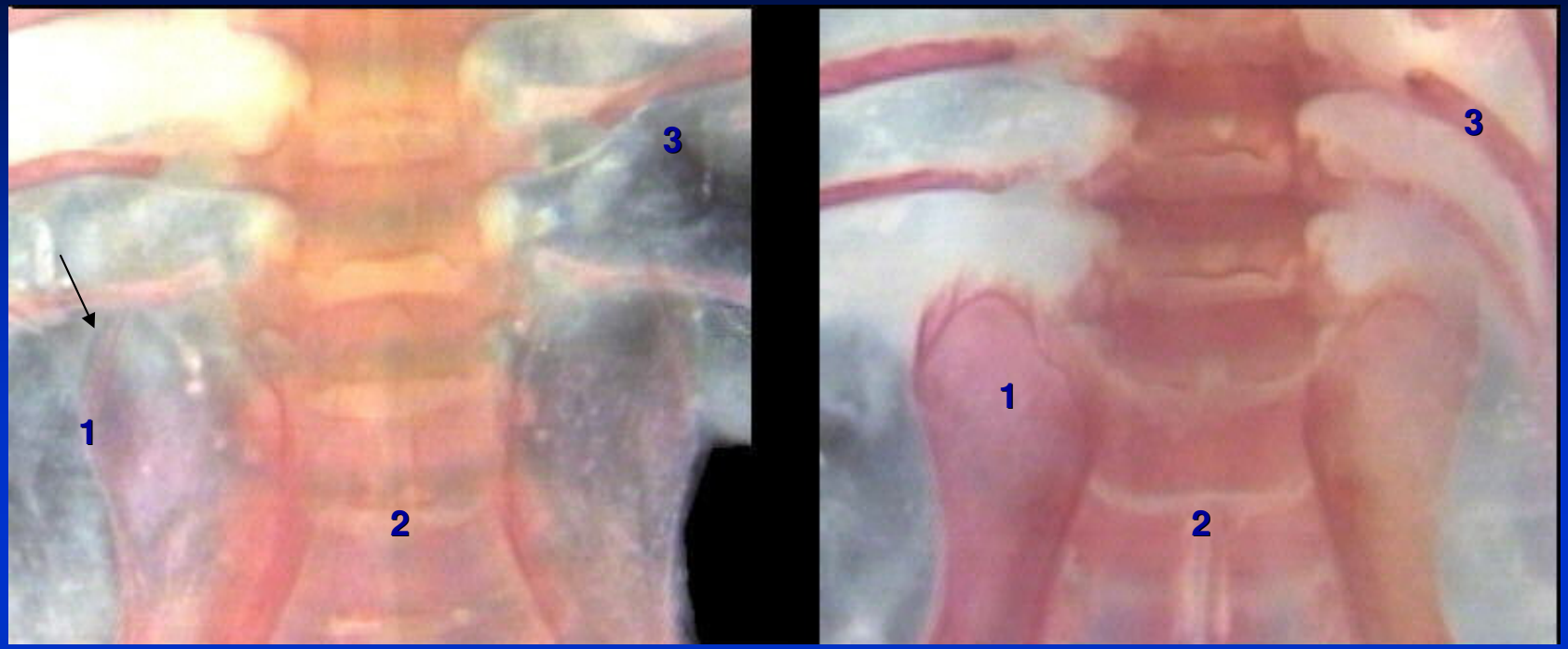
Toes Hyperflexion **Control**

1: Tibiotarsus 2: Tarsometatarsus. 3: Ossa digitorum pedis I.
4: Ossa digitorum pedis II. 5: Ossa digitorum pedis III. 6: Ossa digitorum pedis IV.

Results

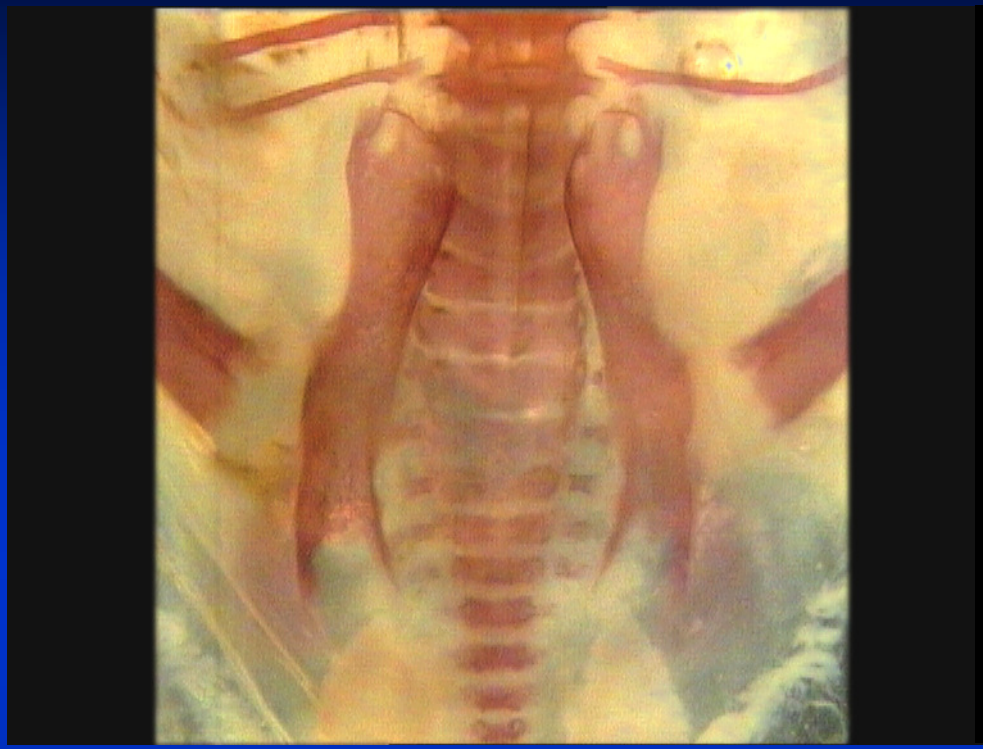
Pelvis skeletal findings

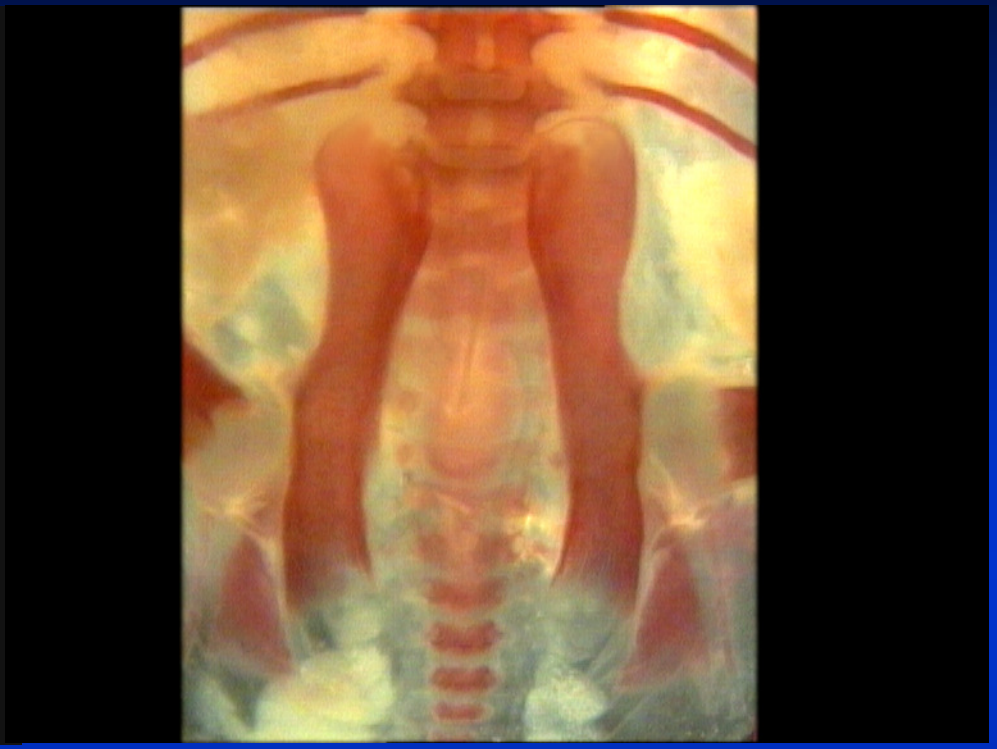
Ilium	incompl.oss.
Ilium	irreg.oss.
Ilium	irreg.spong.
Ilium	poorly oss.
Ischium	incompl.oss.
Os pubis	incompl.oss.
Pelvis	irreg.spong.
Pelvis	poorly oss.



Ilium irregular spongiosa **Control**

1: Os ilium. 2: Lumbosacral vertebra. 3: Costa.





Ilium incomplete ossification

Control

Results

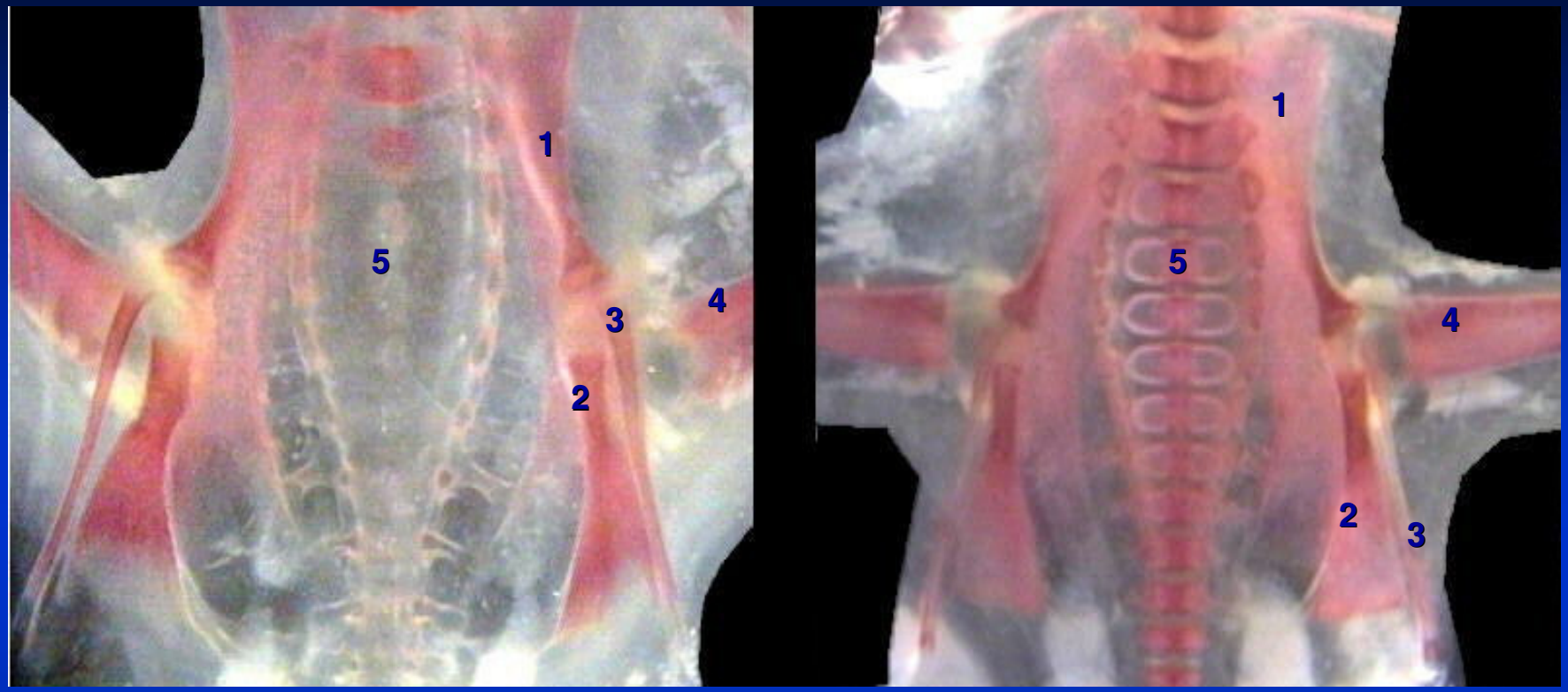
Thorax skeletal findings

Thorax/furcula	bent
Thorax/furcula	irreg. shape
Thorax/furcula	irreg.spong.
Thorax/furcula	O.cent.not os.

Results

Vertebral column skeletal findings

lumb.vertebr.	incompl.oss.
sacr.vertebr.	incompl.oss.
sacr.vertebr.	poorly oss.



Incomplete ossification **Control**

Pelvis (dorsal view). 1: Os ilium. 2: Os ischii. 3: Os pubis. 4: Femur. 5: Lumbosacral vertebra

Conclusion

- The applied method proved suitable for a reliable detection of skeletal anomalies in one-day old quail chicks.
- The evaluation of teratological parameters in birds can be considered as useful in providing additional information for ecotoxicological risk assessment.

Discussion/Questions

- Is the present integration of quail findings into the new terminology suitable?
- If so, classification according to that in mammalian studies?

Discussion/Questions

- Should there be a specific teratology study conducted in avian reproductive toxicity studies?
- If so, is there a suitable timepoint of evaluation comparable to the day of „sectio“?

Discussion/Questions

- If data exist from studies in mammals, should additional specific avian developmental toxicity studies be conducted?

Example: Fentin/TPT

- **<u>Mice:</u> NOAEL < 3.75 mg/kg bw/day** based on fetal skeleton variations (poorly ossified skull bones and vertebrae) and malformations (axis and skull bones) observed in a developmental toxicity study (Sarpa *et al.* (2007).
- **<u>Quail:</u> NOAEL ~ 0.3 mg/kg bw/day** (3 ppm) based on incomplete ossification of pelvic bones and lumbar, sacral and caudal vertebrae (Niemann *et al*., in preparation) as well as reproductive toxicity at 30 ppm (Grote *et al.*, 2006).

References

- Albers, P.H., Hoffman, D.J. and Brisbin, I.I. Jr. (2001) Unusual leg malformations in screech owls from a South Carolina superfund site. *J. Toxicol. Environ. Health*, A, 63, 63-89.

- Chahoud, I., Stahlmann, R., Bochert, G., Dillmann, I., Neubert, D. (1988) Gross-structural defects in rats after acyclovir application on day 10 of gestation. *Arch. Toxicol.,* 62(1),8-14.

- Faqi, A.S., Schweinfurth, H., Chahoud, I. (1997) Determination of the no-effect dose of bis(tri-n-butyltin)oxide (TBTO) for maternal toxicity and teratogenicity in mice. *Cong. Anom.,* 37, 251-258

- Fernie, K., Bortolotti, G. and Smits, J. (2003) Reproductive abnormalities, teratogenicity, and developmental problems in American kestrels (*Falco sparverius*) exposed to polychlorinated biphenyls. *J. Toxicol. Environ. Health*, A, 66, 2089-2103.

References

- Grote, K., Niemann. L., Gericke, C., Selzsam, B. and Chahoud, I. (2006) Effects of fentin hydroxide on reproduction of the Japanese quail (*Coturnix coturnix japonica*). *Environ. Res.,* 101, 81-88.

- Hoffman, D.J. and Albers, P.H. (1984) Evaluation of potential embryoteoxicity and teratogenicity of 42 herbicides, insecticides, and petroleum contaminants to mallard eggs. *Arch. Environ. Contam. Toxicol.*, 13, 15-27.

- Kertész, V., Bakonyi, G. and Farkas, B. (2006) Water pollution by Cu and Pb can adversely affect mallard embryonic development. *Ecotoxicol. Environ. Safety*, 65, 67-73.

- Makris, S.L., Solomon, H.M., Clark, R., Shiota, K., Barbellion, S., Buschmann, J., Ema, M., Fujiwara, M., Grote, K., Hazelden, K.P., Hew, K.W., Horimoto, M., Ooshima, Y., Parkinson, M., Wise, L.D. (2009) Terminology of developmental abnormalities in common laboratory mammals (version 2) *Reprod.Toxicol.,* 28(3),371-434

References

- OECD (1984) Organisation for Economic Co-operation and Development (OECD) guideline 206 for testing of chemicals. Avian reproduction test. Adopted 4 April 1984. OECD Internet homepage (www.oecd.org/document).

- OECD (2000) Organisation for Economic Co-operation and Development (OECD) guideline for testing of chemicals. Proposal for a new test guideline. Avian reproduction toxicity test in the Japanese quail or Northern bobwhite. Revised draft document. OECD Internet homepage (www.oecd.org/document).

- Sarpa, M., De-Carvalho, R.R., Delgado, I.F. and Paumgartten, F.J.R. (2007) Developmental toxicity of triphenyltin hydroxide in mice. *Regul. Toxicol. Pharmacol.*, 49, 43-52.

References

- Stoll, S. (2002): Etablierung einer Methode zur Bestimmung von Skelettanomalien der Japanischen Wachtel (*Coturnix coturnix japonica*). Inaugural-Dissertation (*i.e.*, PhD Thesis), Freie Universität Berlin, Fachbereich Veterinärmedizin (unpublished, available only in German).

- Wise, L.D., Beck, S.L., Beltrame, D., Beyer, B.K., Chahoud, I., Clark, R.L., Clark, R., Druga, A.M., Feuston, M.H., Guittin, P., Henwood, S.M. , Kimmel, C.A., Lindstrom, P., Palmer, A.K., Petrere J.A., Solomon, H.M., Yasuda, M., York, R.G. (1997) Terminology of developmental abnormalities in common laboratory mammals (version 1). *Teratology*. 55(4),249-92.

Thank you for your attention!