Zebrafish in developmental toxicity study

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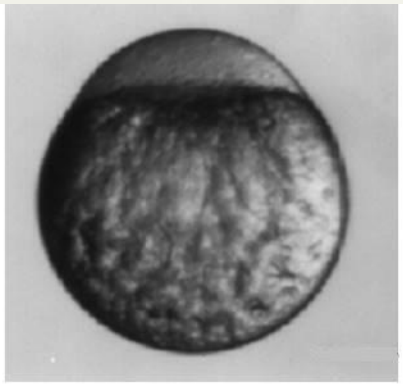
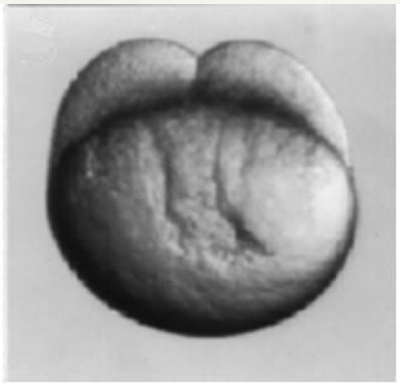
- Introduction of zebrafish
- Zebrafish and teratogenic studies
- Compared zebrafish with mammals in teratogenic studies
- Zebrafish and DBP

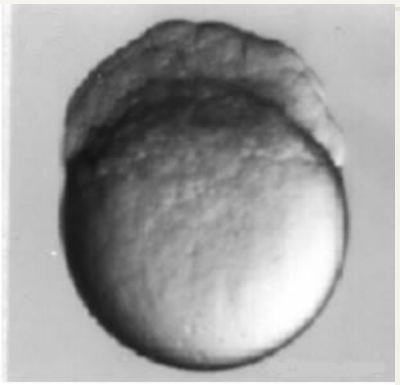
Zebrafish (Danio rerio)

- AB, from a pet shop in Oregon
- HK, from a HongKong fish dealer
- TU, from a Tuebingen pet shop
- WIK, polymorphic TU line

Development stage of zebrafish

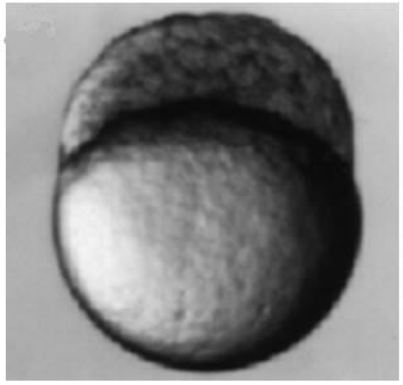


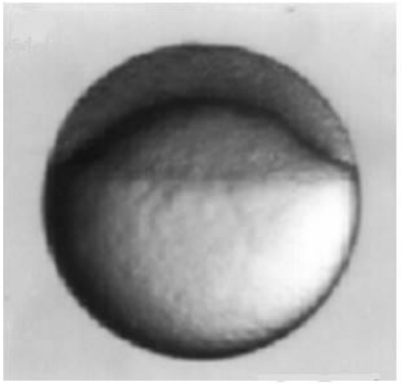




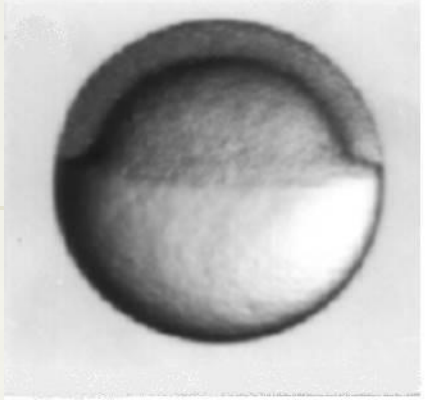
Zygote period: 0- hpf (hour post fertilization)

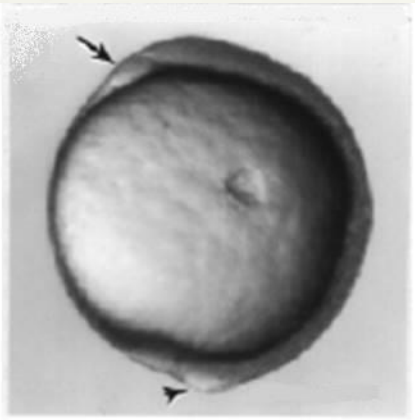
Cleavage period: 0.75- hpf



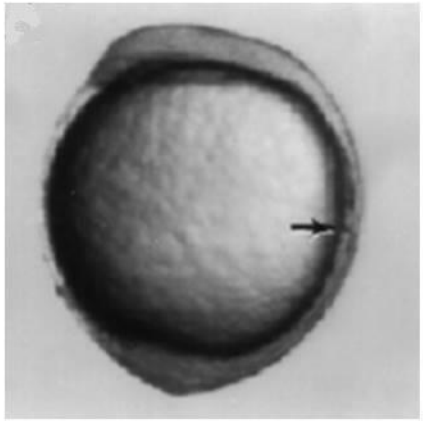


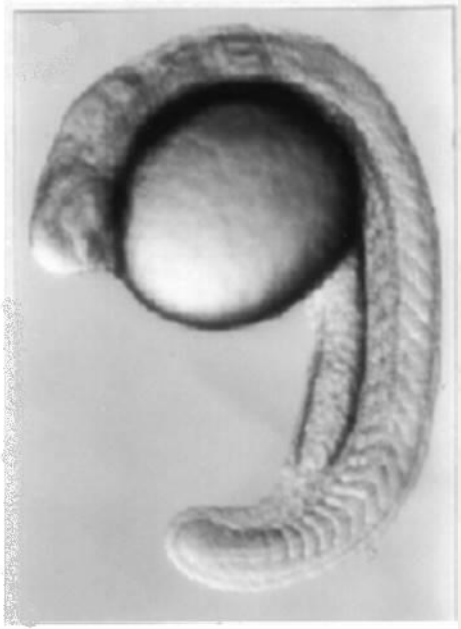
Blastula period: 2.25- hpf





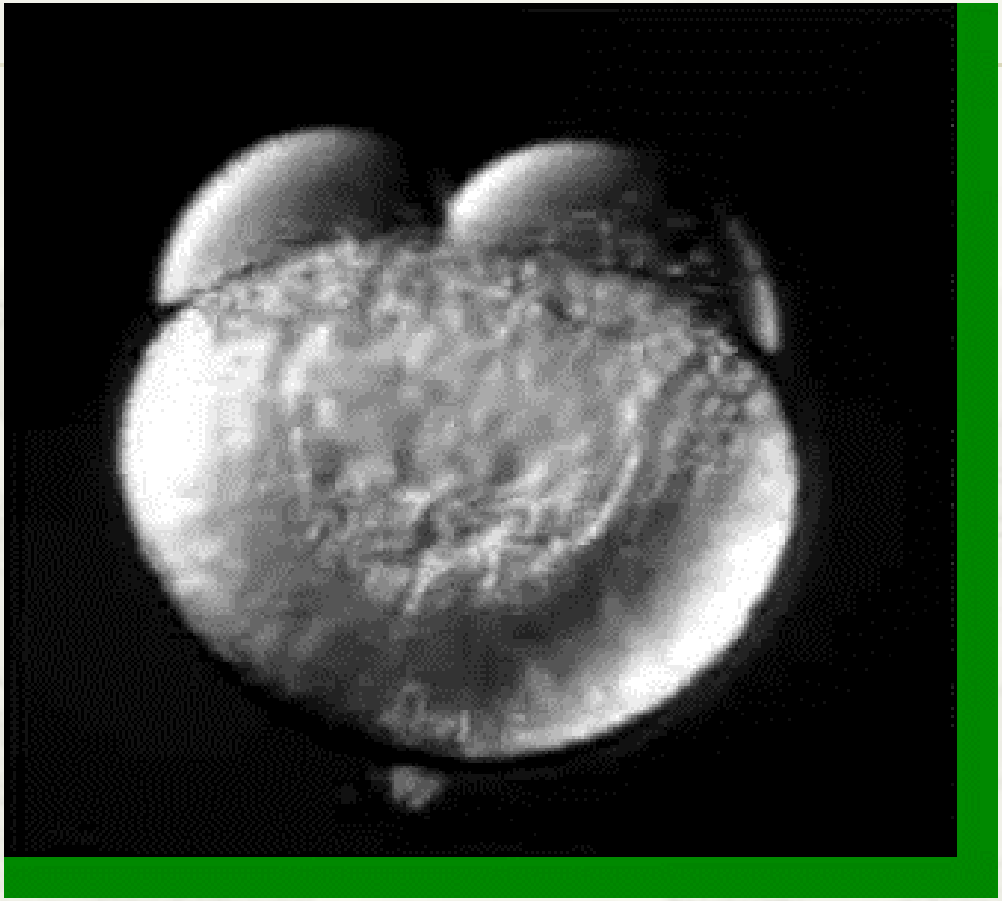
Gastrula period: 5.25- hpf





Segmentation period: 10.3- hpf

Pharyngula period: 24- hpf——Hatching period: 48- hpf



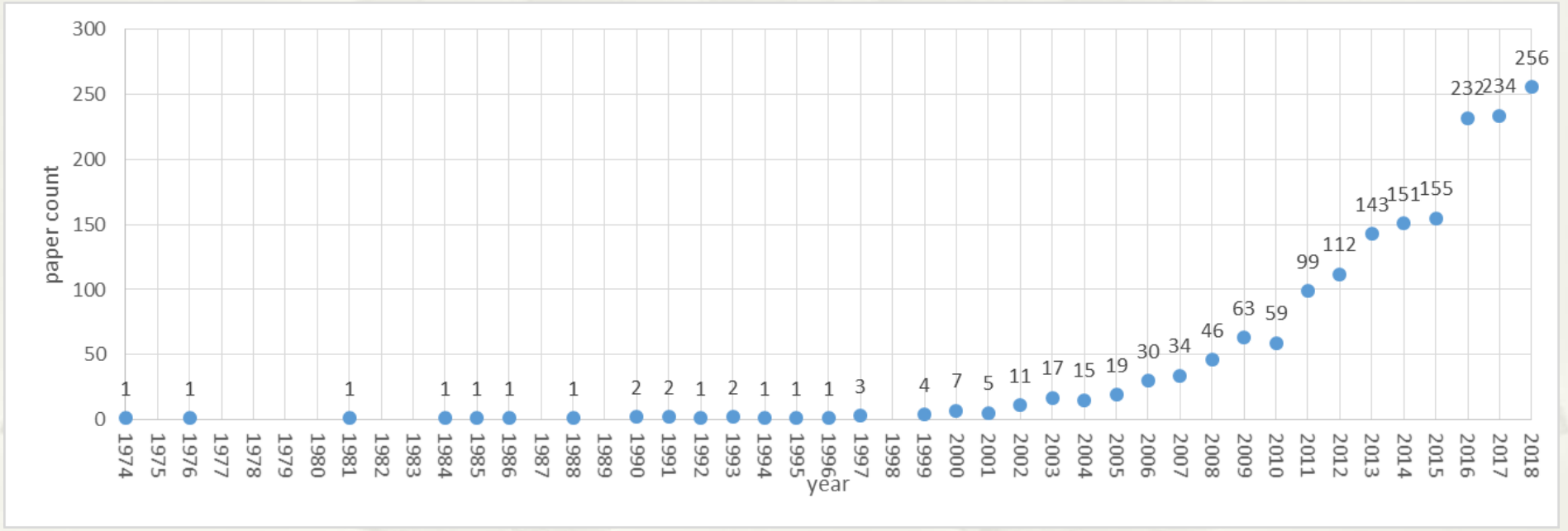
Advantage

- Conserved
  - Vertebrate
  - a full range of cyp genes demonstrate a strong evolutionary relationship
- Simple
  - High fecundity
  - External fertilization and development, chemicals can be added to the medium
  - Small size, suitable for high-throughput screening
- Transparent
  - From fertilization to larval stages
  - unpigmented mutant Casper
  - Transgenic as surveillance tools

Fish toxic test guideline

- OECD guidelines for the testing of chemicals
  - 210: Fish early-life stage toxicity test
  - 215: Fish juvenile growth test
  - 229: Fish short term reproduction assay
  - 236: Fish embryo acute toxicity (FET) test
  - 305: Bioaccumulation in fish aqueous and dietary exposure
- EPA
  - OPPTS 850.1075: Fish acute toxicity test
  - OPPTS 850.1400: Fish early-life stage toxicity test

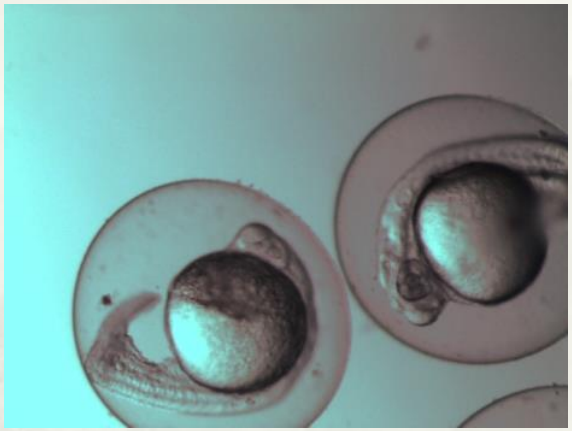
Pubmed database of "zebrafish and toxicity"

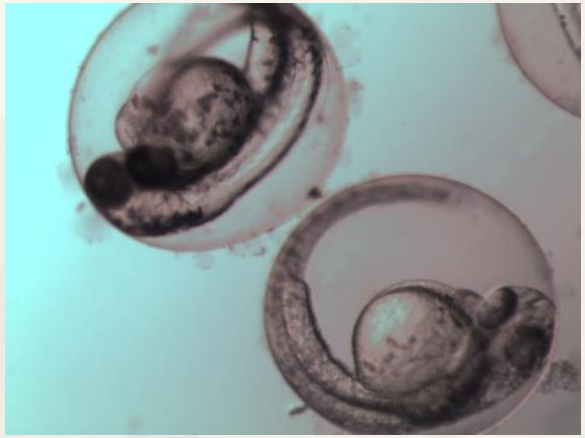


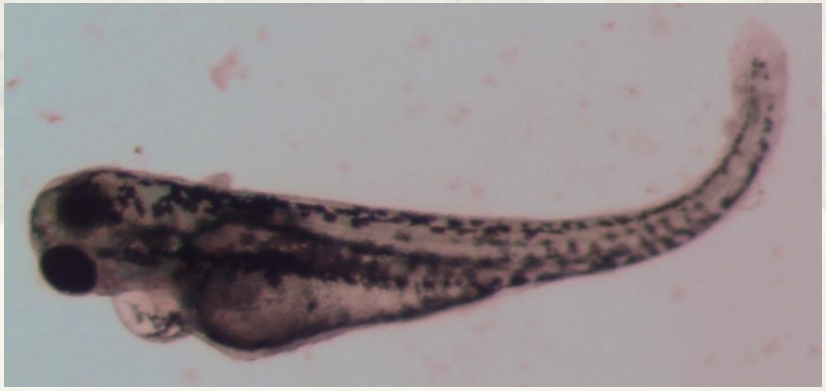
Zebrafish teratogenic

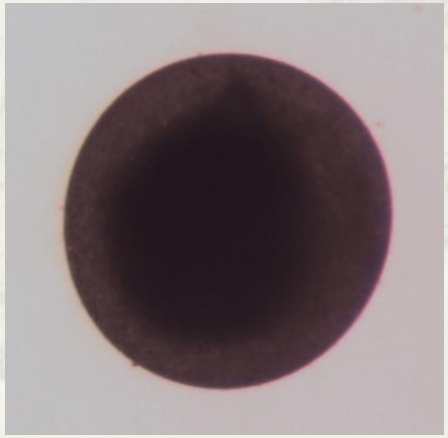
- External findings
  - Anatomical microscope
- Visceral findings
  - Transgenic organ, whole mount of in situ hybridization (Immunofluorescence)
- Skeletal findings
  - Alcian Blue and (or) Alizarin Red

External findings

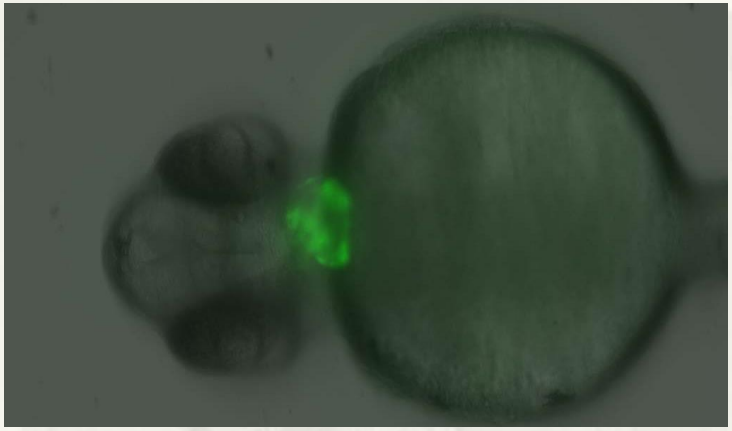


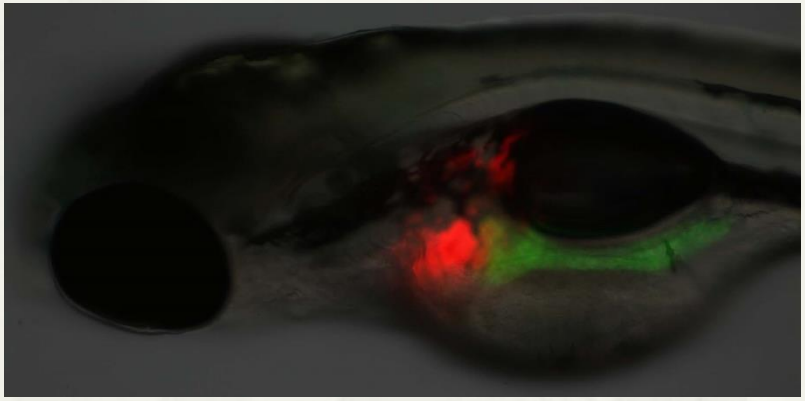




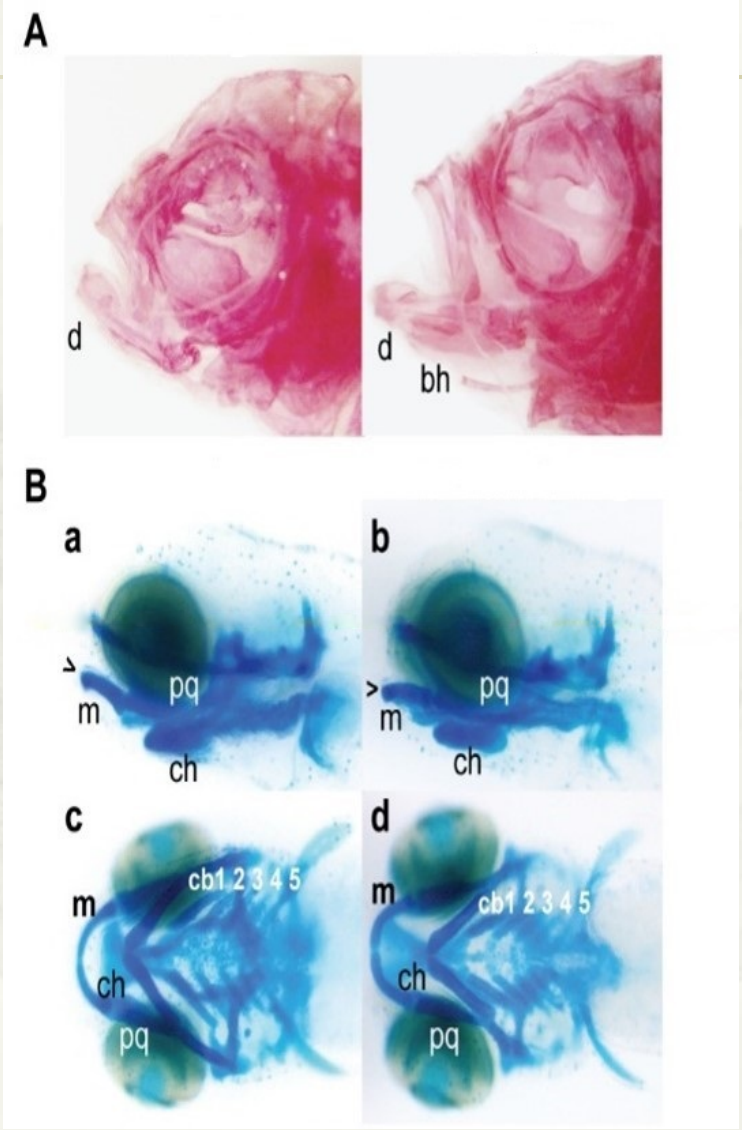


Visceral findings





Skeletal findings



Zebrafish and pesticides

- Until July of 2017, there are 850 publications when searched with key words: zebrafish and pesticide and terato* not environmen*
- There are 48 active substances (AS) mentioned in these papers.
- Check each AS in ECHA and JMPR to get the teratogenic information of mammals.

Teratogenic ASs in both zebrafish and mammals

- 2,4-D.---herbicide /preservative
  - In zebrafish
    - 72hpf, 25mg/l: reduced body length, pericardial edema, The expression of amhc and vmhc were not restricted in atrium and ventricle
  - In rat
    - gavage, 75mg/kg bw, sternbrae malaligned

- Chlopyrifos---pesticide /insecticide
  - In zebrafish
    - 0.4mg/l: curved spines, shortened tails (4dpf), shorten segment (72hpf)
  - In mouse
    - gavage, 25mg/kg bw, delayed ossification;

- Clomazone---herbicide
  - In zebrafish
    - 120hpf: edema (13.4mg/l), lack in gas bladder formation (6.7mg/l), craniofacial deformations (26.8mg/l), tail tip (53.5mg/l) and spine deformations (3.4mg/l)
  - In rat
    - gavage, 300mg/kg bw, delayed ossification, increased hydroureter

- Carbendazim---fungicide
  - In zebrafish
    - 72hpf: pericardial edema, head and spine deformities (1.41mg/l), eye deformities (1.53mg/l), tail deformities (1.66mg/l); 96hpf: pericardial edema (1.19mg/l), spine deformity (1.3mg/l).
  - In rat
    - gavage, 30mg/kg bw, anasarca. exencephalia, meningocele and an abbreviated tail but microphthalmia, internal hydrocephalus, malformations of the ribs, the spine (cleft vertebrae), the sternum, the heart and the lungs;
    - diet, 6000ppm =371mg/kg bw, supernumerary ribs
  - In rabbit
    - the thoracic vertebrae, and the cervical vertebrae.

Tab. 3 LC_{50}, EC_{50} and TI values as derived from the concentration-response curves for 9 compounds at the 144 hpf time points and comparison of classification of compounds based on animal and human versus zebrafish data

Compound	IC_{50} / mol·L^{-1}	EC_{50} / mol·L^{-1}	TI	Mammalian classification [7-10]	Human data classification	Zebrafish classification
ATRA	2.96×10^{-8}	2.86×10^{-9}	10.35	T	T	T
Methimazole	3.28×10^{-3}	1.13×10^{-3}	2.91	T	T	T
Indometacin	1.63×10^{-4}	9.74×10^{-4}	1.67	T	T	T
Acetaminophen	2.84×10^{-3}	1.37×10^{-3}	2.07	T	T	T
Methotrexate	7.52×10^{-2}	5.74×10^{-2}	1.31	T	T	T
5-Fluorouracil	4.28×10^{-5}	5.15×10^{-6}	8.31	T	T	T
Ascorbic acid	/	/	/	N	N	N
Penicillin G	/	/	/	N	N	N
Isoniazid	/	/	/	N	N	N
Saccharin	/	/	/	N	N	N

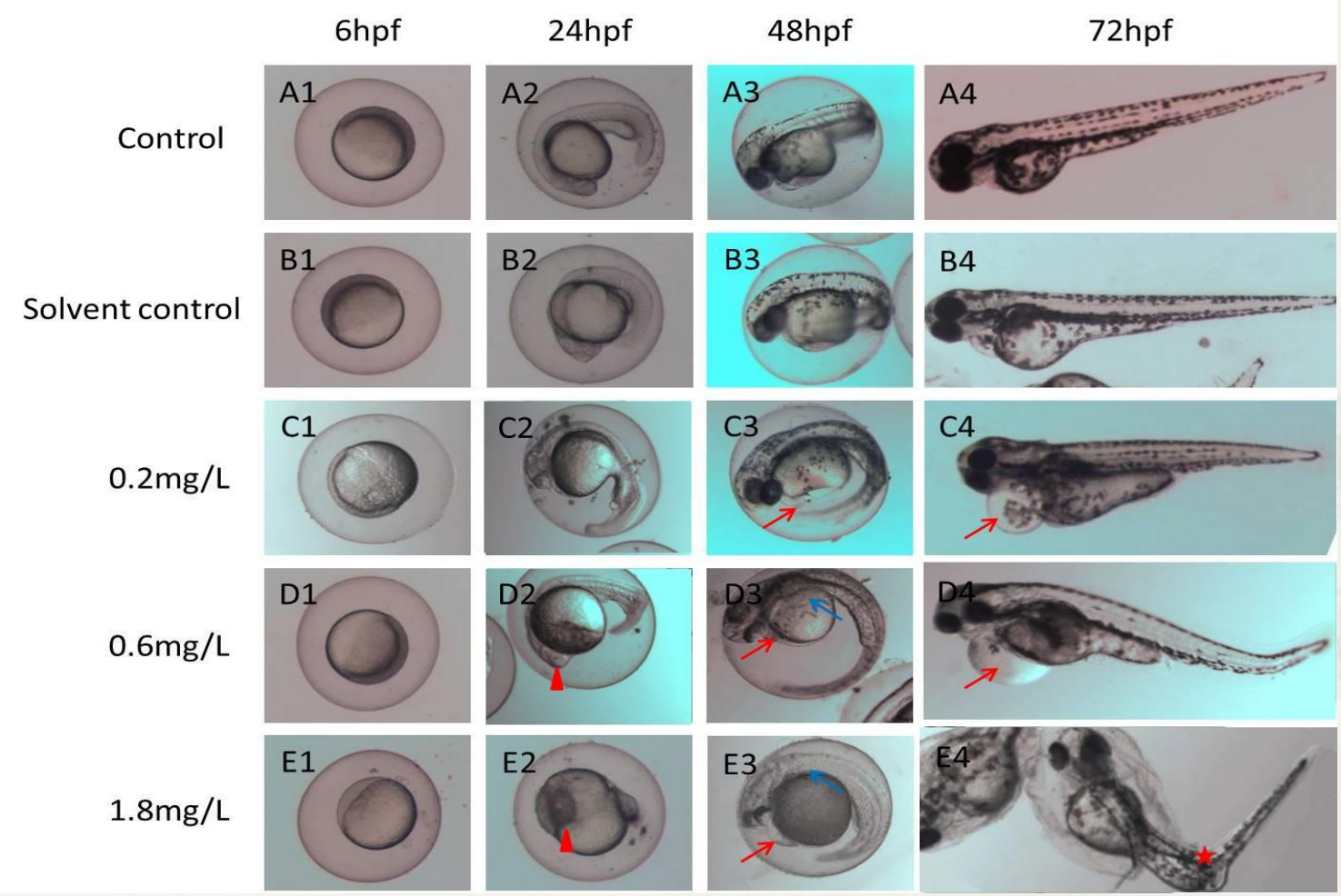
Teratogens (T) and non-teratogens (N) was classified by the TI obtained.

Zebrafish and Dibutyl phthalate (DBP)

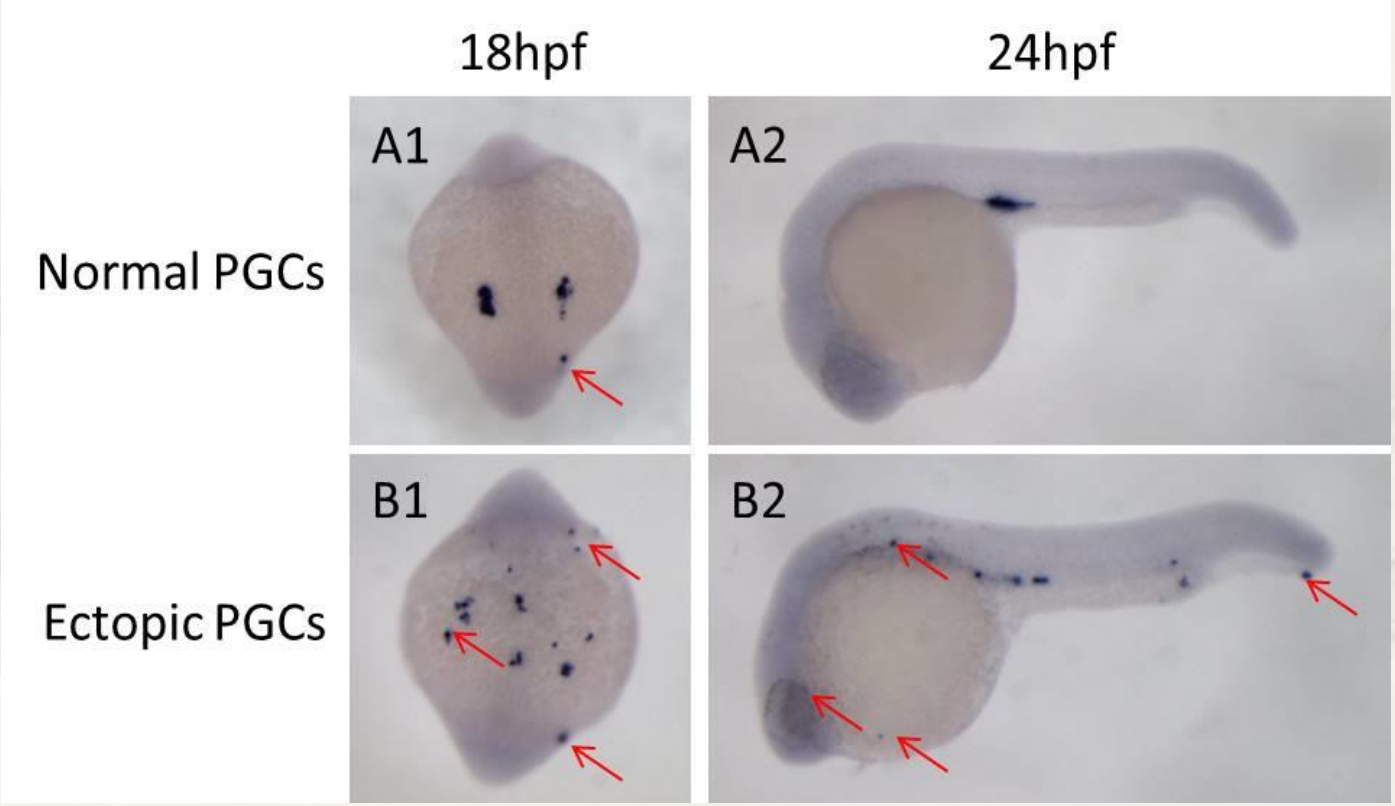
- DBP is an environmental endocrine disrupters (EEDs), used primarily as plasticizers to impart flexibility to polyvinylchloride plastics.

- DBP disturb Sertoli Cell function, disrupt Sertoli-Germ celll interaction, reduce sperm production.
- DBP disturb Leydig Cell function, reduce testosterone.

Zebrafish embryo exposure of DBP



Disterb primordial germ cells (PGCs) distrubution



Conclusion

- Some of the chemicals induce same or at least similar teratogenic effect in zebrafish and in mammals.
- Zebrafish could be a proper candidate to be one of the model organisms in devtox database.

THANK YOU!