

# **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

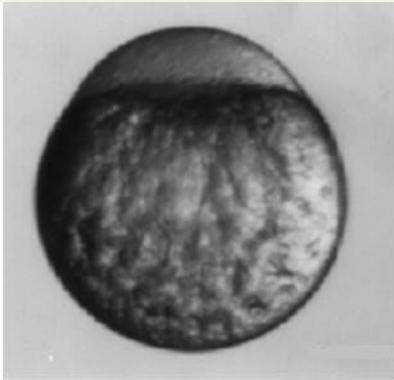
# Zebrafish (*Danio rerio*)

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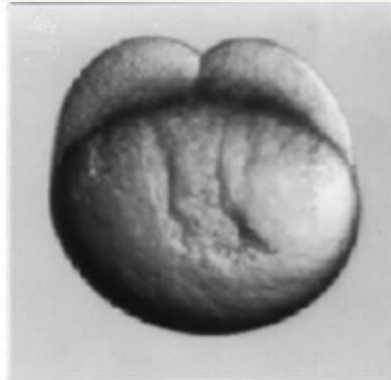
- \* AB, from a pet shop in Oregon
- \* HK, from a HongKong fish dealer
- \* TU, from a Tuebingen pet shop
- \* WIK, polymorphic TU line



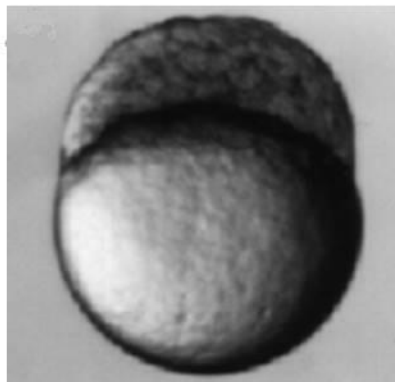
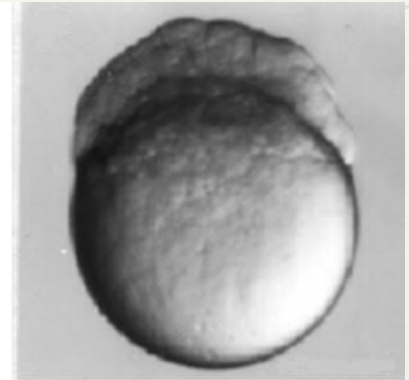
# Development stage of zebrafish



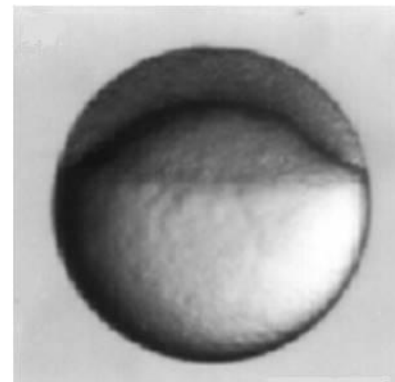
Zygote period: 0- hpf (hour post fertilization)

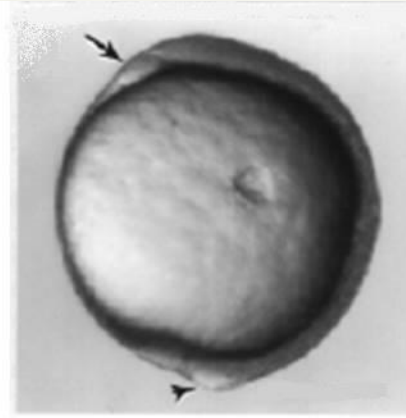
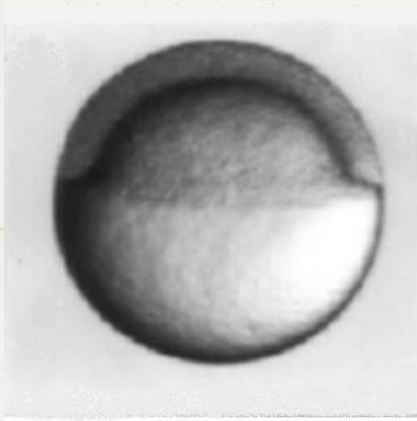


Cleavage period: 0.75- hpf

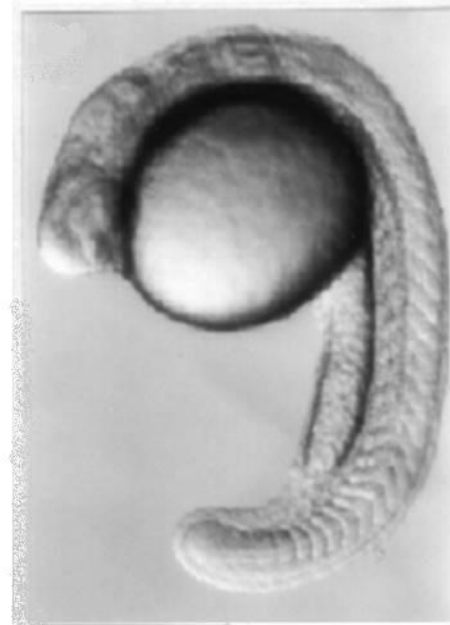
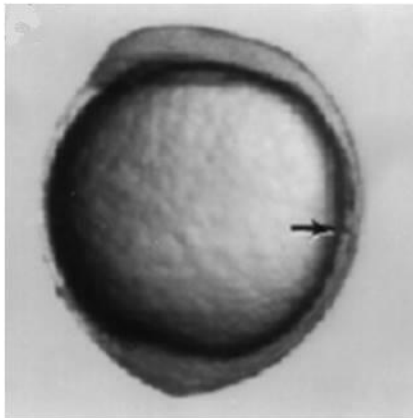


Blastula period: 2.25- hpf





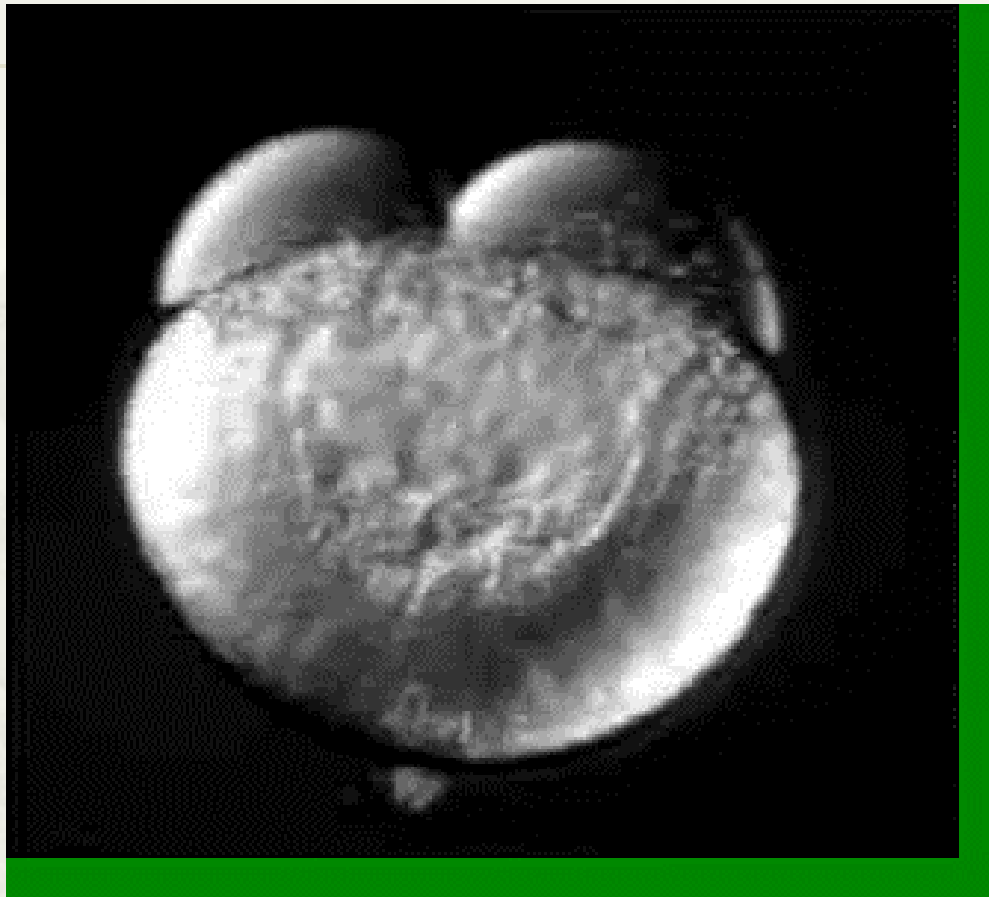
Gastrula period: 5.25- hpf



Segmentation period: 10.3- hpf



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



# Advantage

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- \* 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

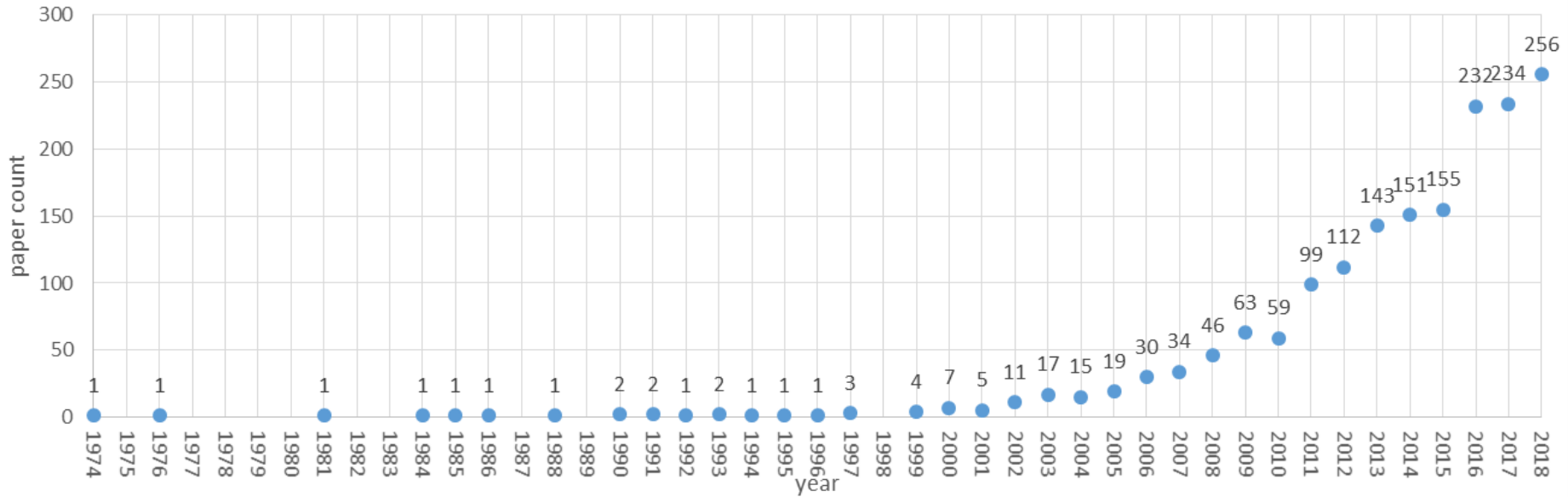


# Fish toxic test guideline

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- \* 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”

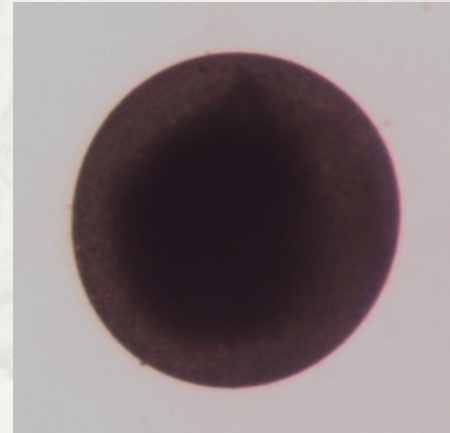
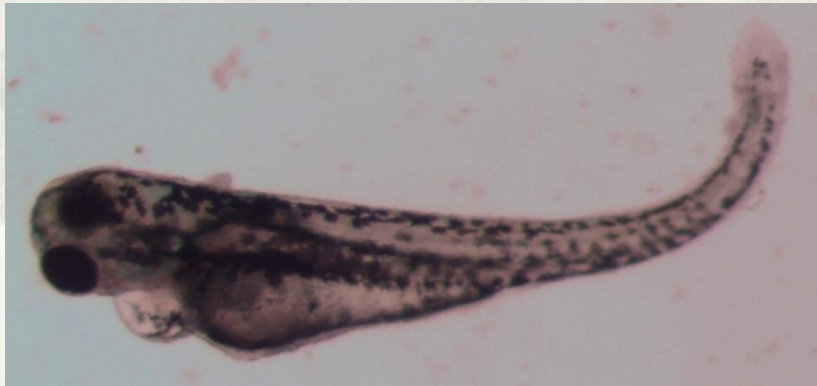
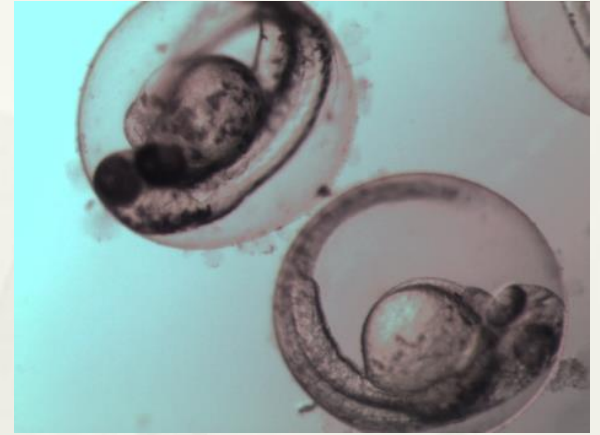
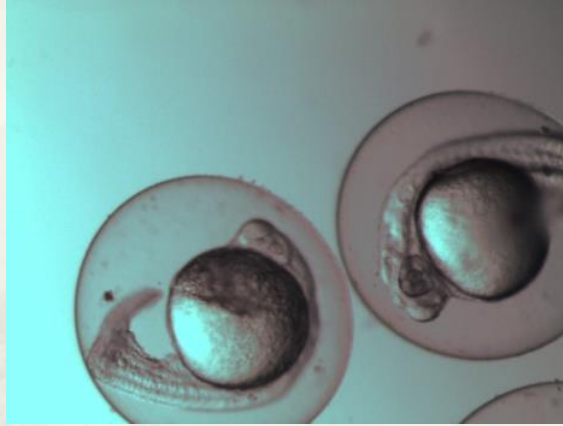


# Zebrafish teratogenic

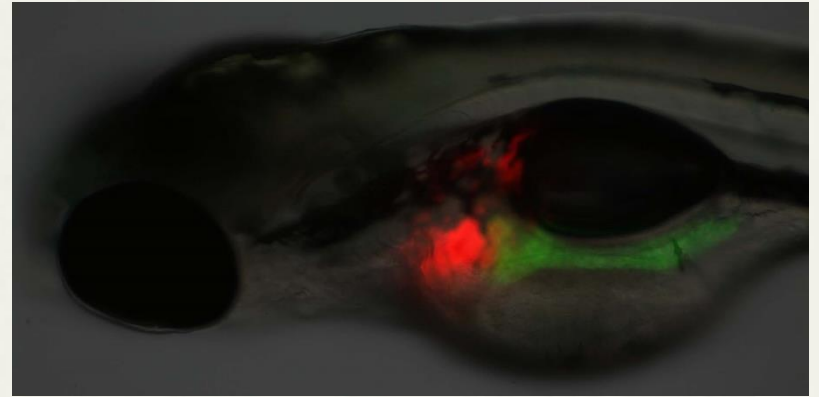
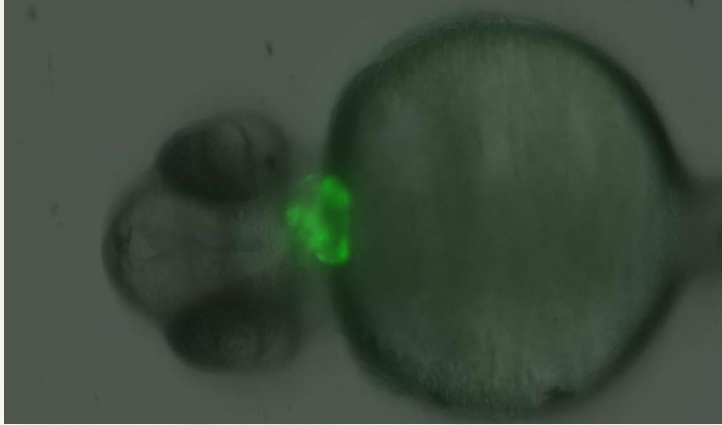
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- \* External findings
  - \* Anatomical microscope
- \* Visceral findings
  - \* Transgenic organ, whole mount of in situ hybridization (Immunofluorescence)
- \* Skeletal findings
  - \* Alcian Blue and (or) Alizarin Red

# External findings

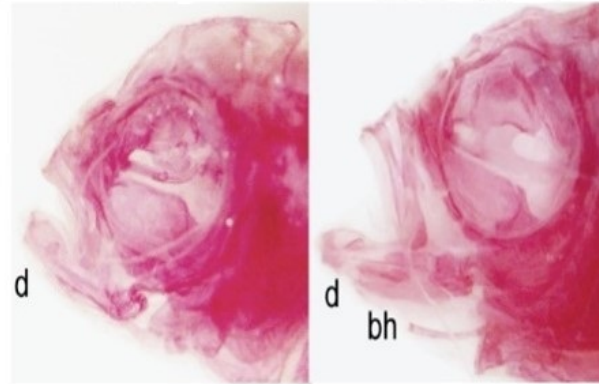


# Visceral findings

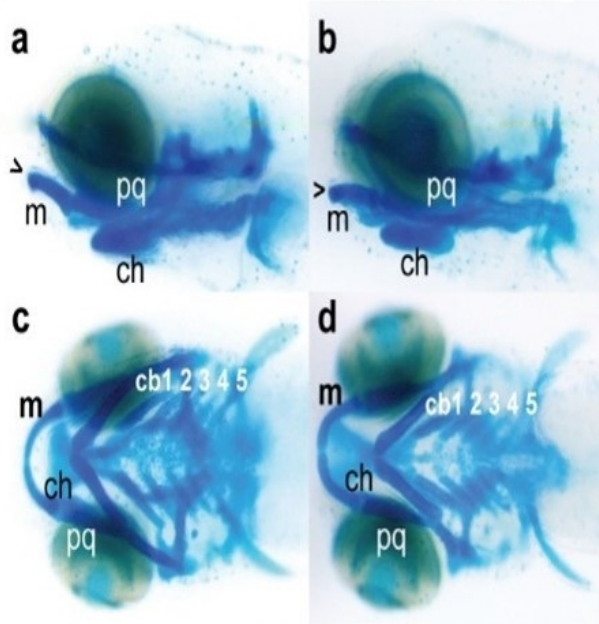


# Skeletal findings

A



B



# Zebrafish and pesticides

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- \* 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

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- \* 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



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- \* 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<sub>50</sub>, EC<sub>50</sub> 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 <sub>50</sub> / mol•L <sup>-1</sup>	EC <sub>50</sub> / mol•L <sup>-1</sup>	TI	Mammalian classification <sup>[7-10]</sup>	Human data classification	Zebrafish classification
ATRA	2.96 × 10 <sup>-8</sup>	2.86 × 10 <sup>-9</sup>	10.35	T	T	T
Methimazole	3.28 × 10 <sup>-3</sup>	1.13 × 10 <sup>-3</sup>	2.91	T	T	T
Indometacin	1.63 × 10 <sup>-4</sup>	9.74 × 10 <sup>-4</sup>	1.67	T	T	T
Acetaminophen	2.84 × 10 <sup>-3</sup>	1.37 × 10 <sup>-3</sup>	2.07	T	T	T
Methotrexate	7.52 × 10 <sup>-2</sup>	5.74 × 10 <sup>-2</sup>	1.31	T	T	T
5-Fluorouracil	4.28 × 10 <sup>-5</sup>	5.15 × 10 <sup>-6</sup>	8.31	T	T	T
Ascorbic acid	/	/	/	N	N	N
Penicillin G	/	/	/	N	N	N
Isoniazid	/	/	/	N	N	N
Saccharin	/	/	/	N	N	N

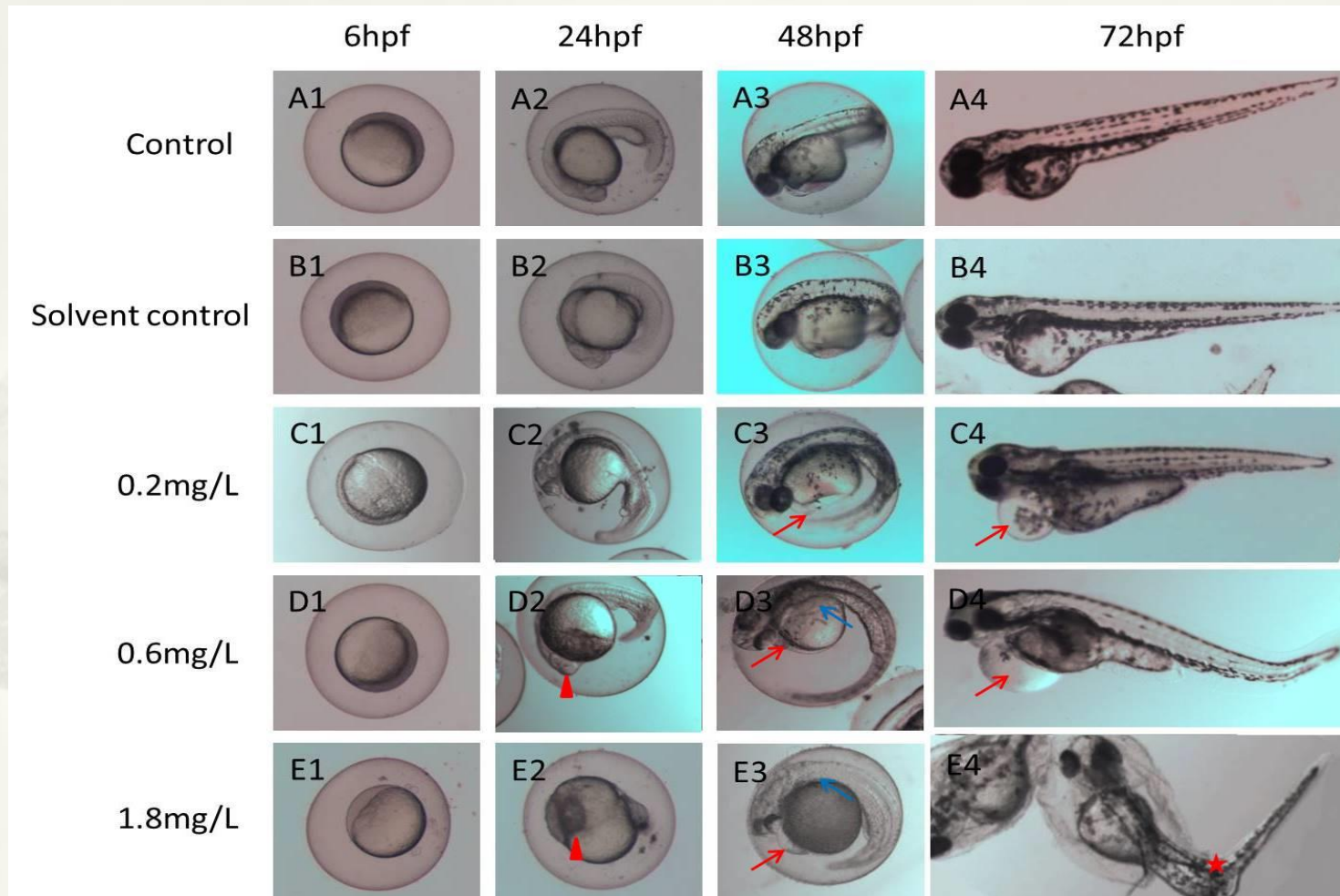
Teratogens (T) and non-teratogens (N) was classified by the TI obtained.

# Zebrafish and Dibutyl phthalate (DBP)

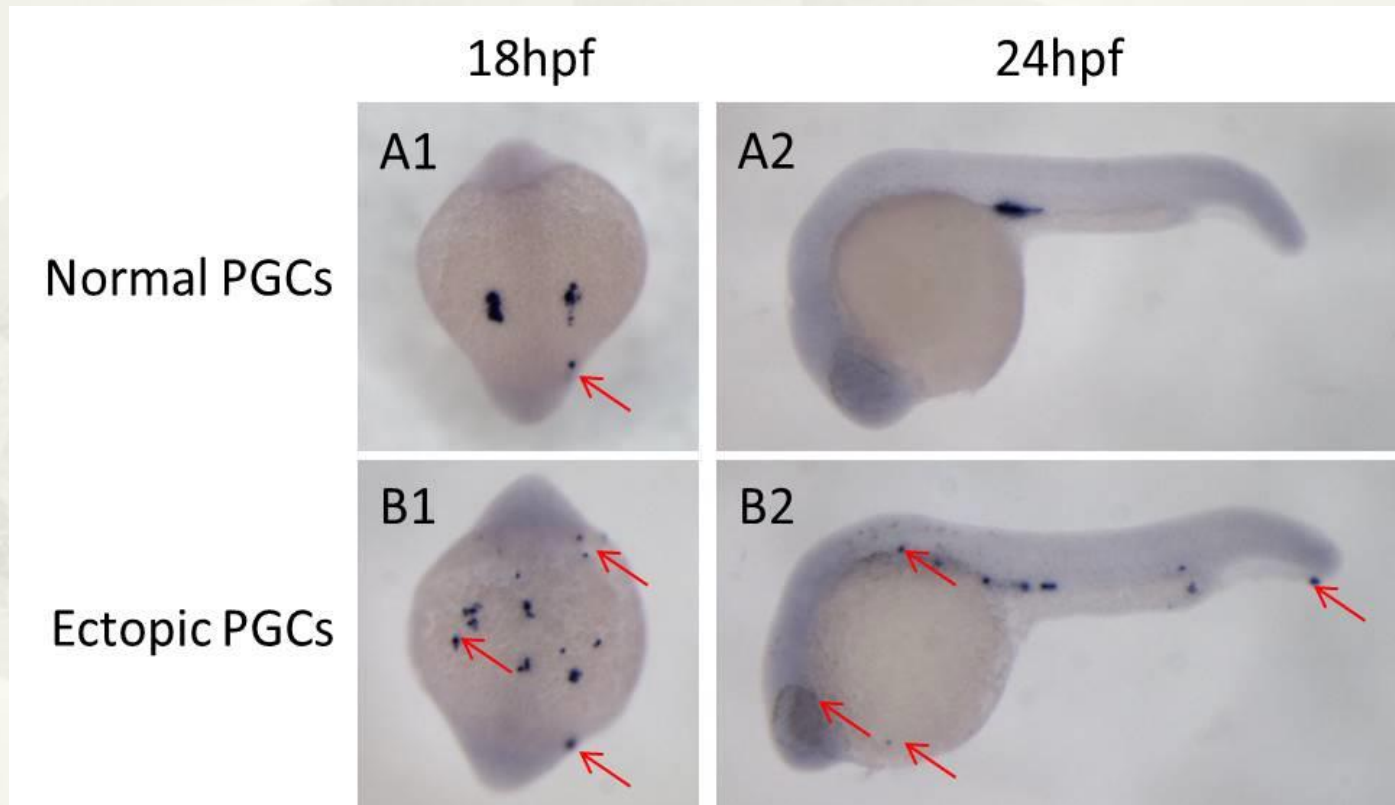
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- \* DBP is an environmental endocrine disruptors (EEDs), used primarily as plasticizers to impart flexibility to polyvinylchloride plastics.
- \* DBP disturb Sertoli Cell function, disrupt Sertoli-Germ cell interaction, reduce sperm production.
- \* DBP disturb Leydig Cell function, reduce testosterone.

# Zebrafish embryo exposure of DBP



# Disturb primordial germ cells (PGCs) distribution



# Conclusion

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- \* 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.



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**THANK YOU!**