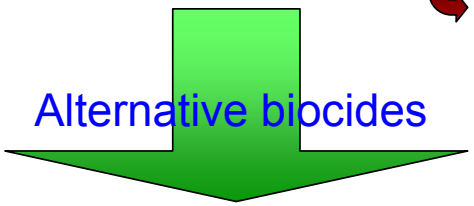
Chronic toxicity of antifouling biocides, copper pyrithione to a marine fish, the mummichog (*Fundulus heteroclitus*)

○Kazuhiko MOCHIDA[1], Katsutoshi ITO[1], Hiroya HARINO[2], Toshimitsu ONDUKA[1], Akira KAKUNO[1] and Kazunori FUJII [1]

1 National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hiroshima, JAPAN
2 Osaka City Institute of Public Health and Environmental Sciences, Osaka, JAPAN

Introduction

- **Antifouling biocide**



Organotin compound (TBT, etc.)

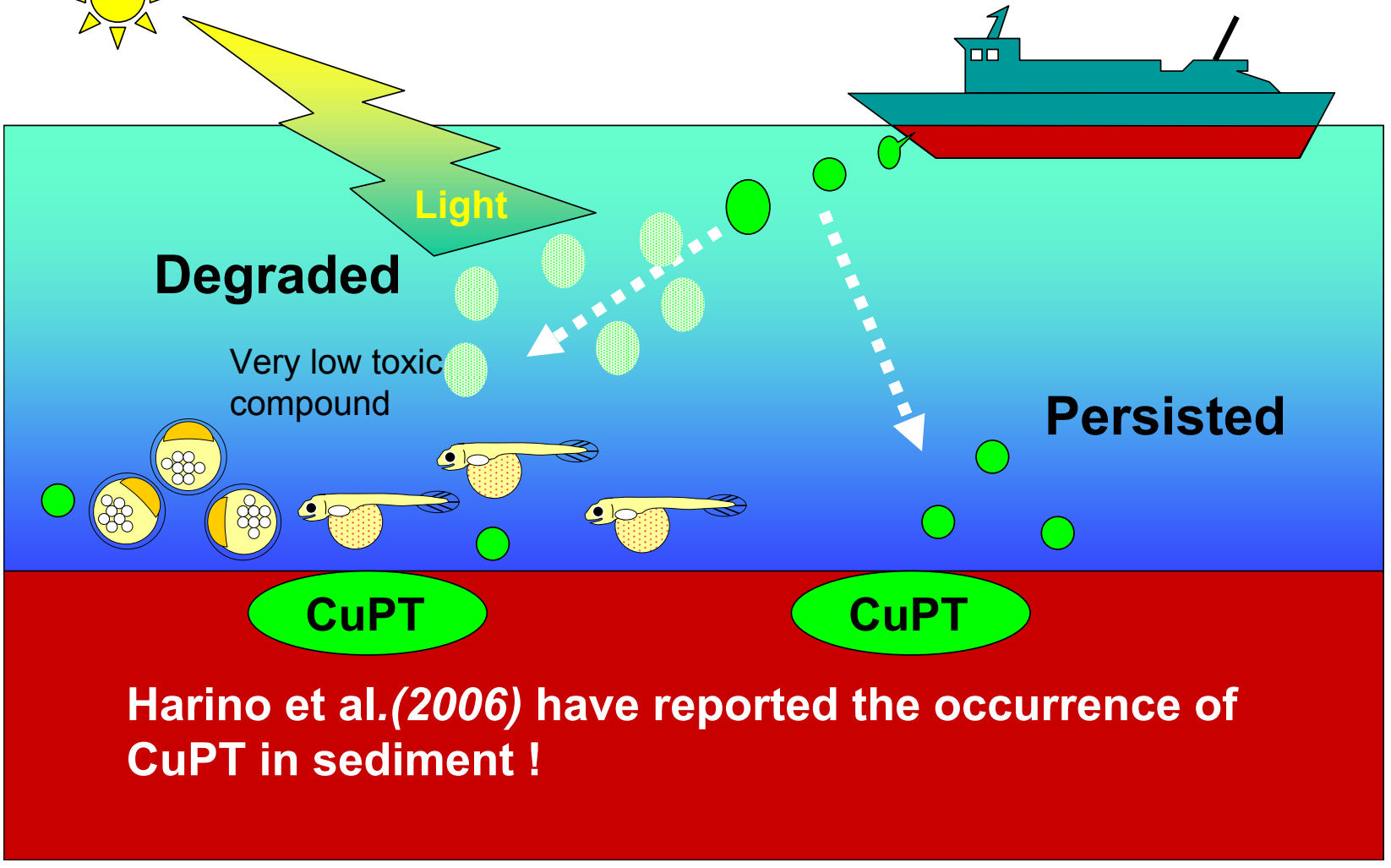
➡ **We have focused on toxicity of**

Metal pyrithione, such as copper pyrithione (CuPT) and zinc pyrithione (ZnPT)

- **The 2nd and 3rd most used biocides in Japan**

(Okamura and Mieno, 2006)

Embryo and larvae of fish could be influenced by the toxicity of the metal pyrithione



Harino et al.*(2006)* have reported the occurrence of CuPT in sediment !

In the present study···

- Chronic toxicity to a marine teleost fish (Early life-stage toxicity test)

- Effect of the metal pyrithione and their photo-degradation products on acetylcholinesterase activity

Early-life toxicity test

【OECD TG 210, etc】

- Mummichog（embryo, late brastula – early gastrula）
- Test chemical ➡ (CuPT)
  0(Cont), 0.5, 1, 2 & 4 μg/L
- Flow-through condition

Water temp., 24.5±0.2 ℃
pH, 7.6
DO, 6.1±0.1 mg/L

- Dark (24h)

Fertilized egg
（embryo）

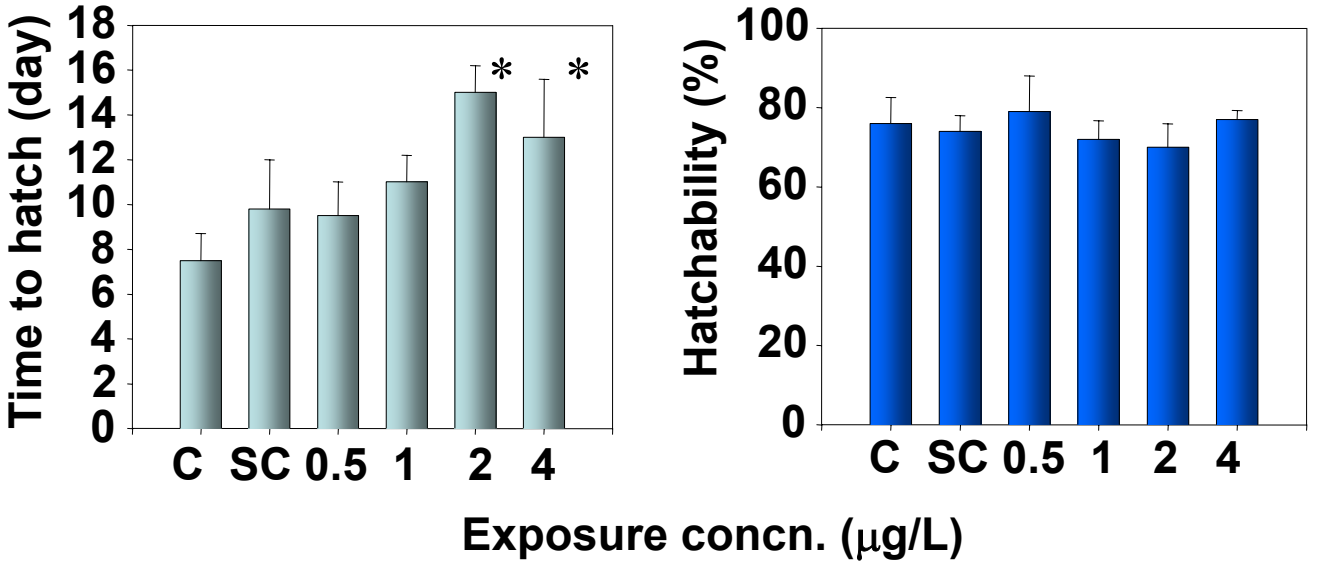
0 — 14d — 21d — 50d

- Time to hatch
- Hatchability

- Growth
- Survival
- Abnormality

Water analysis
CuPT ➡ LC-MS/MS

Effect of long-term exposure of CuPT on time to hatch and hatchability of mummichog



C, control; SC, solvent control

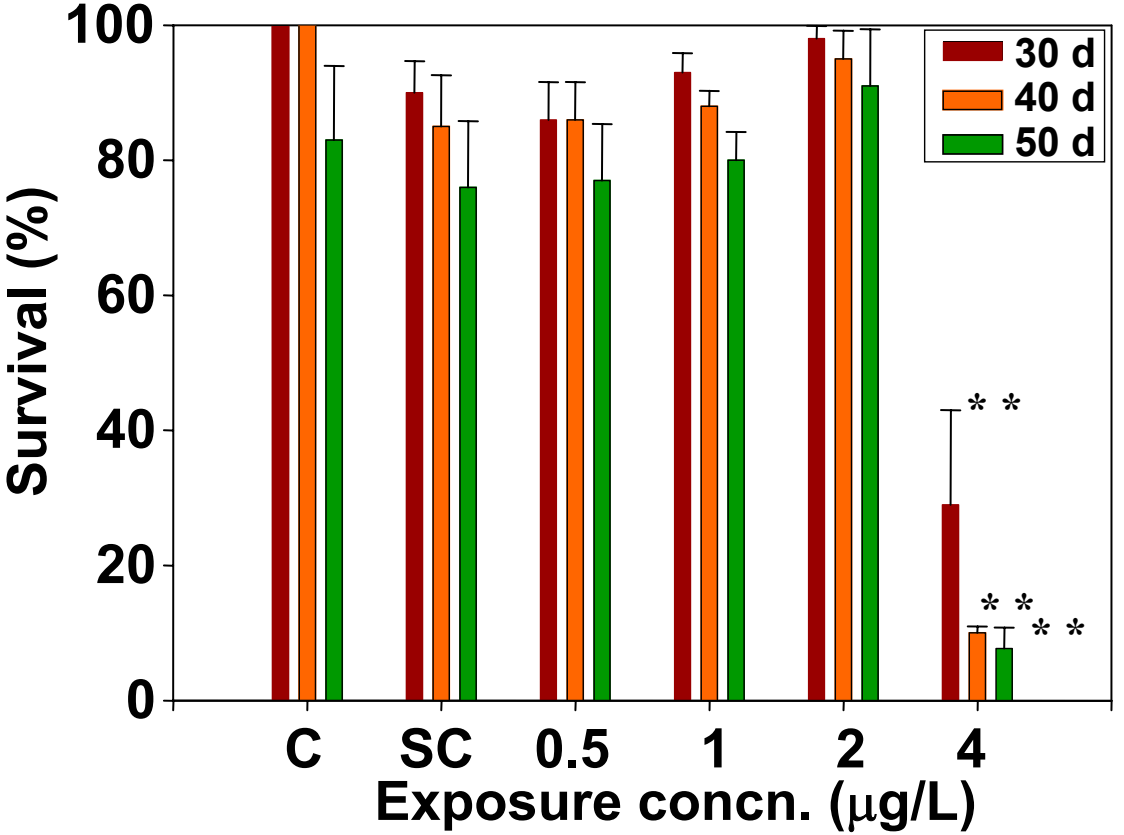
* Significantly (p<0.05) different from value for control

Effect of 50-d exposure of CuPT on growth of mummichog

Concn. (μg/L)	n	Total length (mm)	Body weight (mg)
Control (0)	51	30±0.7	302±21
Solvent Cont.	58	32±0.5	368±15
0.5	50	30±0.7	319±20
1	56	31±0.5	308±16
2	64	25±0.8	209±19
4	52	16±3.0	55±11

Data significantly ($p<0.01$) different from value for control

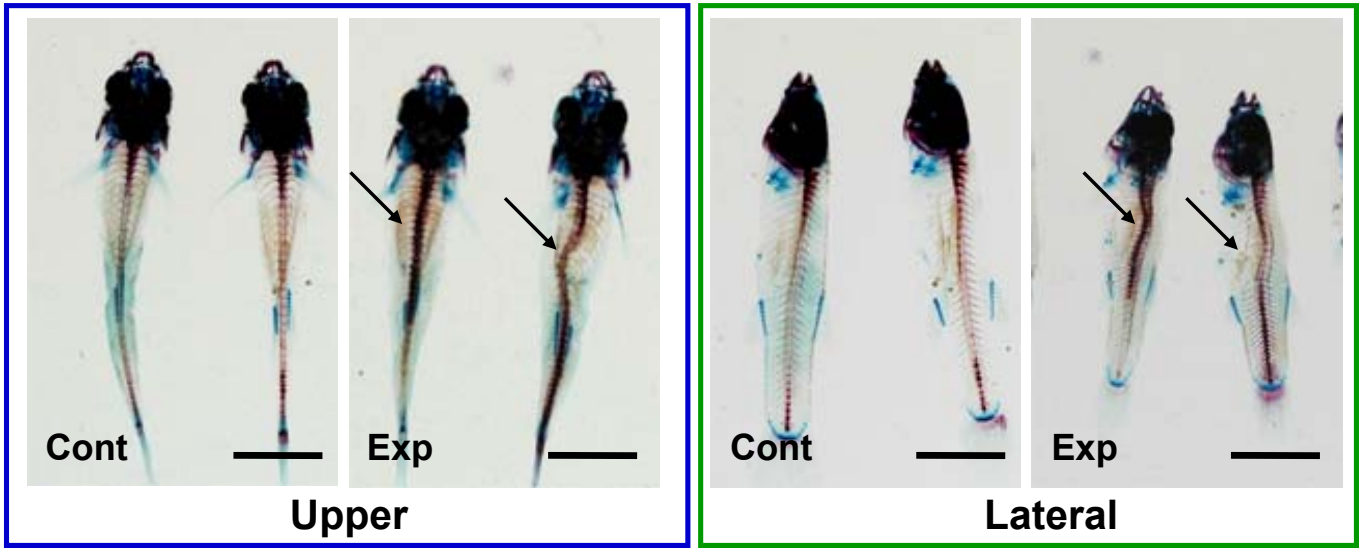
Effect of 50-d exposure of CuPT on survival of mummichog



C, control; SC, solvent control

** Significantly (p<0.01) different from value for control

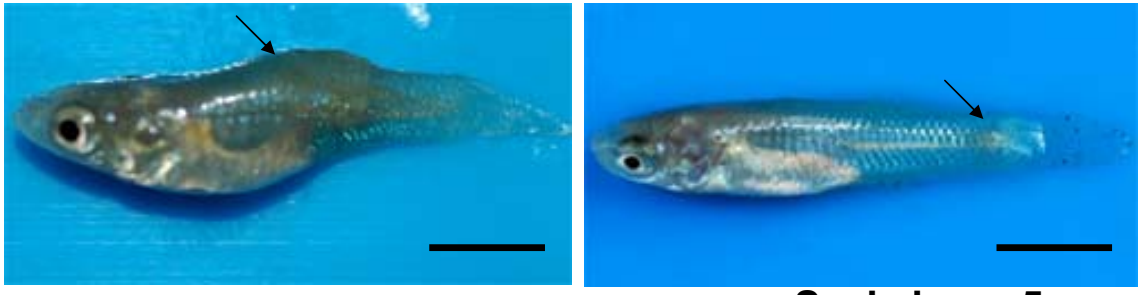
Vertebral deformity induced by the exposure to CuPT



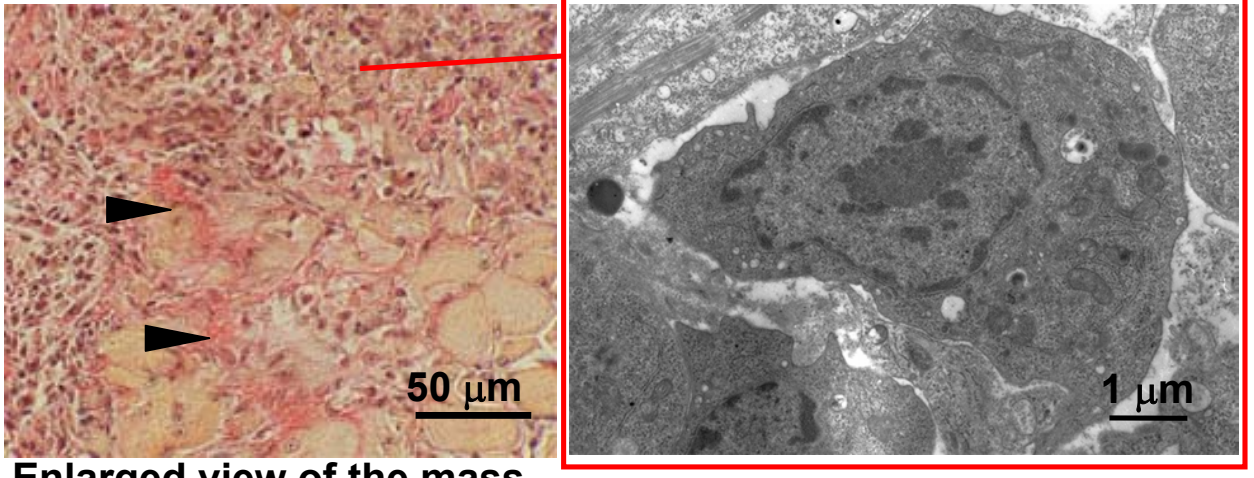
Scale bars、0.5 mm

Ref. Vertebral deformity was also induced in medaka and zebrafish by ZnPT-exposure (Goka, 1999; Sánchez-Bayo and Goka, 2005)

Inflammatory mass induced by the exposure to CuPT



Scale bars, 5 mm



Enlarged view of the mass

Morphological abnormality (%) induced by long-term exposure of CuPT

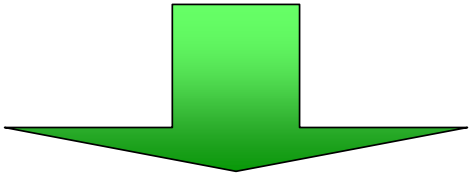
Concn. (μg/L)	Duration of exposure		
	30 d	40 d	50 d
Control (0)	0	0	0
Solvent Cont	0	0	0
0.5	0	0	0
1	0	0	0
2	0	4.6±5.3	5.5±6.4
4	0	75±29	100±0

Index of abnormality

➡ Scoliosis or inflammatory mass formation, or both

The early life-stage toxicity test

- Time to hatch and growth are the most sensitive parameters.



The lowest observed effect concentration (LOEC) ⟶ 2 μg/L（actual concn. 0.37 μg/L）

The no observed effect concentration (NOEC) ⟶ 1 μg/L（actual concn. 0.24 μg/L）

- **Effect of the metal pyrithione and their photo-degradation products on acetylcholinesterase activity**

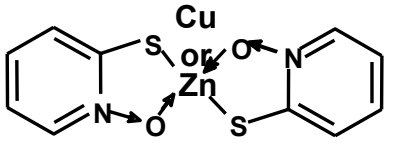
Organophosphorous pesticide

↓ Inhibition of acetylcholinesterase activity (neuro-signal blocking)

Induce vertebral deformity

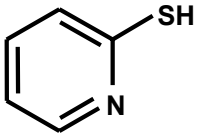
Degradation products of metal pyrithione

- **CuPT, ZnPT**



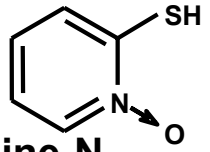
- **HPS**

  (2-mercaptopyridine)



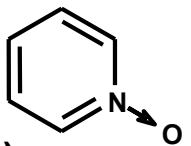
- **HPT**

  (2-mercaptopyridine-N-oxide)



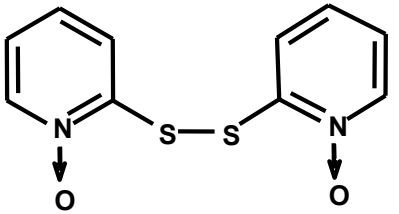
- **PO**

  (Pyridine-N-oxide)



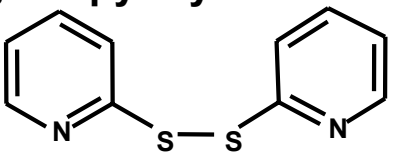
- **(PT)2**

  (2,2'-dithio-bis-pyridine-N-oxide)



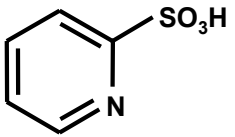
- **(PS)2**

  (2,2'-dipyridyl disulfide)

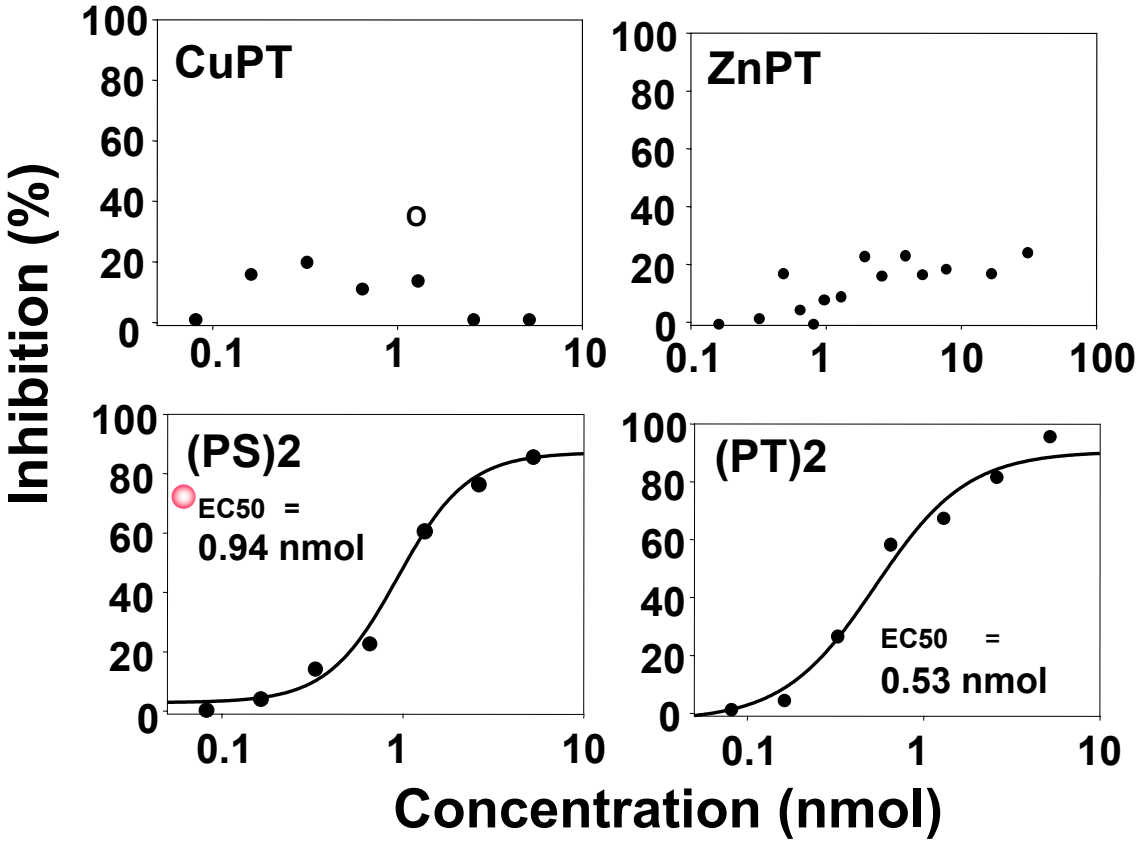


- **PSA**

  (Pyridine-2-sulfonic acid)

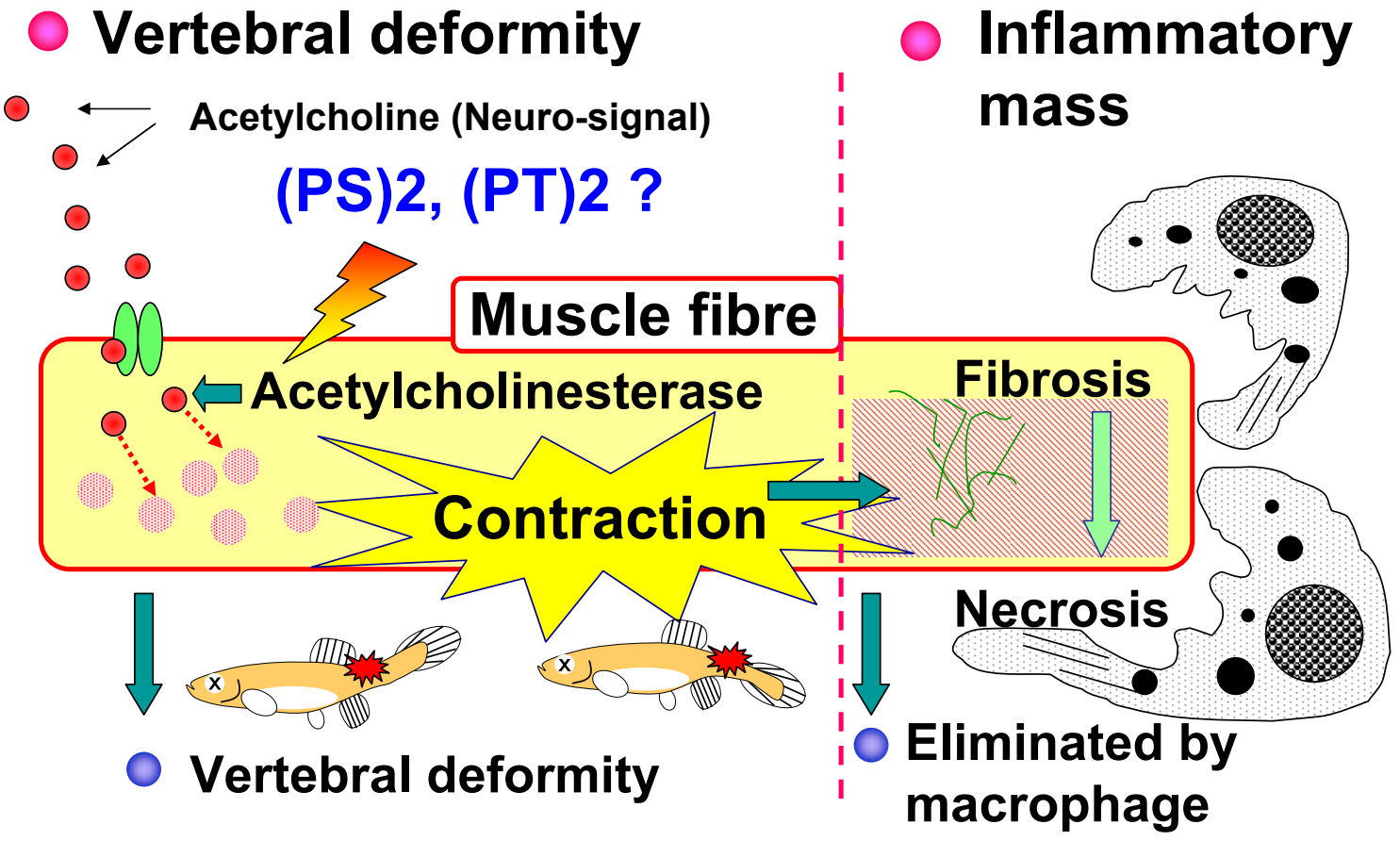


Inhibition assay for acetylcholinesterase activity



- Measured by the method described previously (Ellman et al., 1961) with a slight modification
- Acetylcholinesterase from bovine erythorocyte (5 munits)

Mechanism of the induction of the morphological abnormality



Summary

- Based on toxic effects on time to hatch, growth and survival,

LOEC → 2 μg/L（actual concn. 0.37 μg/L）
NOEC → 1 μg/L（actual concn. 0.24 μg/L）

- Long-term exposure of CuPT induced vertebral deformity and inflammatory mass

Possible mechanism

→ Neuro-muscular blocking properties of (PS)2 and/or (PT)2