Toxic effects of long-term exposure to ibuprofen in *Daphnia magna, Moina macrocopa* and *Oryzias latipes*

Sunyoung Han[1*], Jungkon Kim[1], Sunmi Kim[1], Xiaowei Zhang[2], John P Giesy[2], Kyungho Choi[1]

[1]School of Public Health, Seoul National University, Seoul, 110-799, Korea
[2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, SK S7N 5B3, Canada

Abstracts

- **Chronic exposure to IB could affect survival of fish at the level as low as 1 μg/L, which can be detected in the water environment in extreme events.**
- **We also demonstrated potential endocrine disruption capacity of IB using vitellogenin induction in male fish, and estradiol production and aromatase activity in H295R cells.**

Introduction

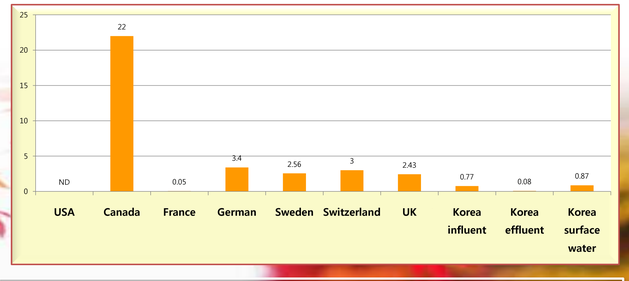
- **Ibuprofen (IB)** is one of non-steroidal anti-inflammatory drugs (**NSAIDs**), and is widely used as analgesic, antipyretic and anti-inflammatory purposes to relieve symptoms of arthritis, rheumatic disorders and fever.
- IB has been **detected frequently in surface water**.
- A number of studies have reported toxic effects of IB on aquatic organisms. However, there is still **paucity of chronic toxicity information.**
- This study was conducted to identify the **effects of IB on steroidogenesis.** In addition, we evaluated the **effects of chronic exposure** of **freshwater crustaceans** (***Daphnia magna*** **and** ***Moina macrocopa***) and **fish** (***Oryzias latipes***) to environmentally relevant concentrations of ibuprofen.

Materials & Methods

- **Chemical:** Ibuprofen (IB, CAS No.: 15687-27-1)
- **Steroidogenesis assay using H295R cells**
  - **Hormone production** - Concentrations of 17β-estradiol (E2) and testosterone(T) were quantified by competitive ELISA using Cayman Chemical® hormone EIA kits.
  - **Aromatase activity in H295R cells** - Aromatase activity was determined by the rate of conversion of 1β-^{3}H-androstenedione to estrone using by LS 6500 multipurpose scintillation counter(Beckman Coulter, Fullerton, USA).
- **Test organisms and toxicity tests**

Organisms	Test type	Test methods
Daphnia magna	Acute test [48h] Chronic test [21d]	US EPA (2002) OECD TG 211 (2008)
Moina macrocopa	Acute test [48hr] Chronic test [7d]	US EPA (2002) Oh (2007)
Oryzias latipes (Japanese medaka)	Chronic test [132d]	enhancement of the OECD TG 210 (1992)

❖ The maximum concentration reported in effluent of sewage treatment plants (STPs) (unit: ug/L)



Reference : USA (Glen R. Boyd, 2003), Canada (Guy L. Brun, 2006), France (C. M. Coetsier, 2009) , German (Thomas A. Ternes, 1998), Sweden (Saioa Zorita, 2009), UK (Martin J. Hilton, 2003), Korea (NIER, 2006)

Results

1. Hormone production and aromatase activity in H295R cells

Hormone production

(a) Estradiol (b) Testosterone

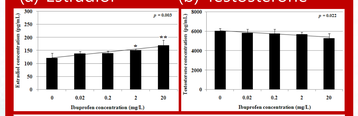


Fig 1. Mean concentrations of (a) estradiol and (b) testosterone in H295R cells after 48 h exposure to ibuprofen

Aromatase activity

(a) Indirect assay (b) Direct assay

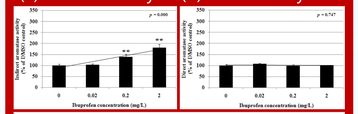


Fig 2. Induction of aromatase activity by ibuprofen in (a) indirect and (b) direct assay

- Ibuprofen exposure increased 17β-estradiol (E2) production and aromatase activity in H295R cells.
- Testosterone (T) Production decreased In a dose-dependent manner.

2. Effects in *D. magna* and *M. macrocopa*

Table 1. Effects of ibuprofen exposure on survival and reproduction for *D. magna* and *M. macrocopa*

Species	Conc. (mg/L)	Adult survival (%)	First day of reproduction	No. of young per female	No. of young per brood	PGR
D. magna	Control	100	10.00±0.00	157.10±10.67	39.28±2.67	0.39
	1.23	100	11.00±1.25	130.80±17.18*	34.77±5.46	0.36
	3.70	100	10.10±0.32	120.20±24.02*	30.84±4.66*	0.35
	11.1	100	11.30±1.49*	111.6±18.73*	31.23±5.14*	0.34
	33.3	100	11.20±1.14*	75.50±27.57*	21.73±3.80*	0.32
	100	0	NA	NA	NA	NA
M. macrocopa	Control	90	3.00±0.00	69.20±11.65	16.88±2.56	0.90
	3.125	100	3.00±0.00	66.40±12.43	18.05±1.70	0.92
	6.25	100	3.00±0.00	69.50±12.65	17.89±2.50	0.92
	12.5	100	3.00±0.00	60.20±16.12	16.37±1.79	0.91
	25.0	70	3.00±0.00	52.70±20.97	16.03±3.18	0.90
	50.0	80	3.00±0.00	50.70±11.96*	18.08±2.97	0.91

3. Effects in *O. latipes*

Table 2. Survival NOEC of ibuprofen on hatchability and survival of fry, juvenile and adult medaka

	Hatchability	Fry survival 7 dph	Juvenile survival 30 dph	Adult survival 90 dph	Adult survival 120 dph
NOEC	>1000	>1000	100	1	0.1

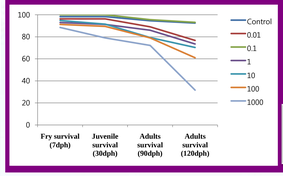


Fig 3. Effects of ibuprofen exposure on hatchability and survival of fry, juvenile a nd adult medaka

Table 3. Effect of ibuprofen exposure on reproduction profile of medaka

Concentration (μg/L)	Surviving pairs	No. of total eggs		No. of broods per pair		No. of eggs Per brood	
		N	mean± s.d.	N	mean± s.d.	N	mean± s.d.
Control	4	4	243.8±64.9	4	28.5±1.0	4	8.5±2.1
0.01	4	4	155.5±48.1	4	24.8±6.0	4	6.3±1.3
0.1	4	4	212.0±51.1	4	26.5±1.9	4	7.9±1.4
1	3	3	283.3±79.0	3	25.0±6.9	4	11.1±1.1
10	4	4	365.5±90.1	4	27.8±2.5	4	13.0±2.3*
100	3	3	285.7±114.0	3	23.7±6.8	4	11.8±1.6*
1000	2	2	256.5±248.2	2	21.0±9.9	3	5.9±0.2

Vitellogenin in male

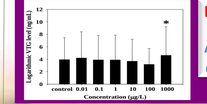


Fig 4. Blood VTG levels in male *Oryzias latipes* across ibuprofen treatments for 120 dph
Asterisk indicates a notable difference from the control ($P < 0.1$)

2nd generation effects

(a) Fertility (b) Hatchability (c) Time to hatch

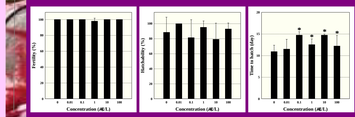


Fig 5. Fertility, hatchability, and time to hatch of progeny generation (F1) observed for 20 d in control water