# Does chiral inversion change the ecotoxicity of pharmaceuticals?

Peta A. Neale[a], Amos Branch[b], Stuart J. Khan[b], Frederic D.L. Leusch[a]

[a]Australian Rivers Institute, School of Environment and Science, Griffith University, Queensland 4222, Australia
[b]School of Civil & Environmental Engineering, UNSW Sydney, New South Wales 2052, Australia

✉ p.neale@griffith.edu.au

## Introduction

- Many pharmaceuticals, including non-steroidal anti-inflammatory drugs (NSAIDs), are chiral chemicals (Fig 1) and their enantiomers can exhibit different biological activity

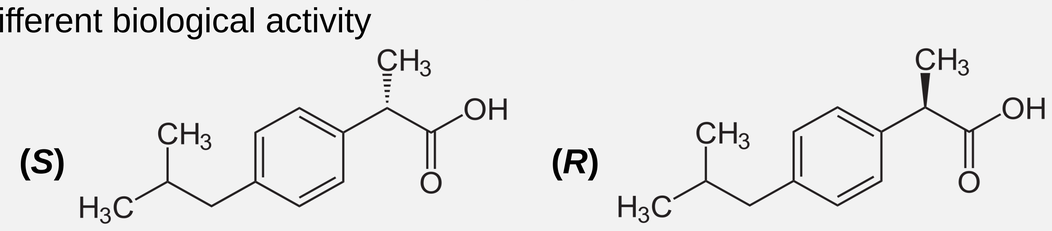


***Fig 1.*** *(S) and (R)-ibuprofen*

- Despite chiral NSAIDs being commonly detected in treated wastewater and surface water, few studies have considered the potential ecotoxicological differences of NSAID enantiomers

**The current study aimed to evaluate the enantiospecific differences of NSAIDs naproxen, ibuprofen, ketoprofen and flurbiprofen using ecologically relevant bioassays with bacteria, algae and fish cells.**

## Methods

- (*S*)- and (*R*)-enantiomers of naproxen, ibuprofen, ketoprofen and flurbiprofen were run in assays indicative of cytotoxicity, photosystem II (PSII) inhibition and induction of cytochrome P450 1A enzymes (CYP1A)
  - Bacterial toxicity assessed using Bacterial Luminescence Toxicity Screen (BLT-Screen) with *Photobacterium leiognathi* [1]
  - Cell viability assessed using neutral red assay with fish liver carcinoma cell line PLHC-1 [2]
  - PSII inhibition assessed using Imaging Pulse Amplitude Modulation (IPAM) fluorometry with green algae *Pseudokirchneriella subcapitata* [3]
  - Ethoxyresorufin-O-deethylase (EROD) activity assessed in PLHC-1 cells after 6 h exposure [4]
- Effect concentration (EC) values were calculated from concentration-effect curves, with the ratio of $EC_{(S)-}$ to $EC_{(R)-}$ determined for each chemical

## Cytotoxicity

- None of the chemicals decreased cell viability in the neutral red assay
- In contrast, all studied enantiomers inhibited bacterial bioluminescence in a dose-dependent manner (Fig 2)
- The naproxen enantiomers were the most toxic, with $EC_{50}$ values of 0.93 and 0.75 mg/L for the (*S*)- and (*R*)-enantiomer, respectively
- There was little difference in effect between the (*S*)- and (*R*)-enantiomers, with the exception of flurbiprofen ((*S*)-enantiomer was more toxic)

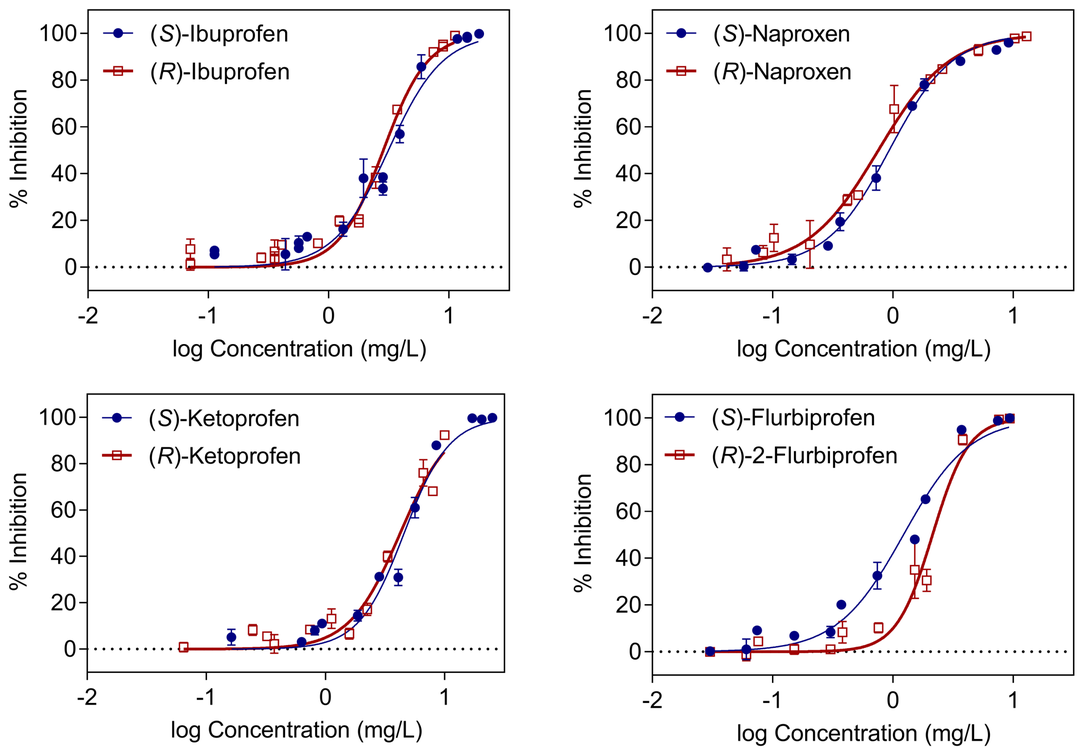


***Fig 2.*** *Log-sigmoidal concentration-effect curves for the enantiomer pairs of ibuprofen, naproxen, ketoprofen and flurbiprofen in the BLT-Screen, with the (S)-enantiomer shown in blue closed symbols and the (R)-enantiomer shown in red open symbols*

## PSII Inhibition

- Only (*R*)-naproxen, (*S*)-flurbiprofen and (*R*)-flurbiprofen induced PSII inhibition in green algae at 2 and 24 h
- The (*R*)-enantiomer was more toxic than the (*S*)-enantiomer for flurbiprofen (Fig 3), but the difference was less than a factor of 2
- This is contrary to the bacterial toxicity results for flurbiprofen, where (*S*)-flurbiprofen was more toxic, indicating species-specific differences in enantiomer potency for flurbiprofen

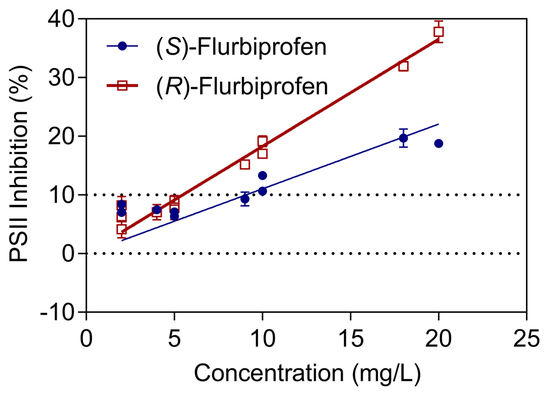


***Fig 3.*** *24 h PSII inhibition linear concentration-effect curves for the flurbiprofen enantiomer pairs*

## EROD activity

- Both naproxen enantiomers induced EROD activity, with (*S*)-naproxen 2.5 times more potent than (*R*)-naproxen ($EC_{IR1.5}$ 5.1 vs 12.6 mg/L, respectively)
- In contrast, only the (*R*)-enantiomers of ketoprofen ($EC_{IR1.5}$ 4.3 mg/L) and flurbiprofen ($EC_{IR1.5}$ 8.4 mg/L) were active in the EROD assay

## Ecological risk of NSAID enantiomers

- The (*S*)- to (*R*)- EC ratio ranged between 0.40 to 1.42 for all assays (Table 1), indicating less than an order of magnitude difference between enantiomers
- This suggests that the risk of overlooking the effect of more potent NSAID enantiomers is minor for the studied bioassays and that using racemic effect data is sufficient for risk assessment purposes
- However, a (*S*)- to (*R*)- EC ratio of at least 6.3 was estimated for ketoprofen in the EROD assay, suggesting further investigation may be warranted

***Table 1.*** *$EC_{(S)-}/EC_{(R)-}$ for the studied NSAIDs*

| Chemical | Bacterial Toxicity | PSII Inhibition (24 h) | EROD activity |
|---|---|---|---|
| Ibuprofen | 1.10 | *not active* | *not active* |
| Naproxen | 1.24 | 1.2* | 0.40 |
| Ketoprofen | 1.08 | *not active* | >6.3* |
| Flurbiprofen | 0.57 | 1.42 | >1.5* |

*$EC_{(S)-}/EC_{(R)-}$ estimated as only the (*R*)-enantiomer had an effect for naproxen, ketoprofen and flurbiprofen

- The effect of the enantiomer pairs was compared to the combined effect based on literature enantiomeric fractions for each NSAID in wastewater effluent [5-8] (Fig 4)
- In most cases, the combined EC value fell between the effect of each enantiomer pair, with the exception of naproxen
- Naproxen is manufactured as (*S*)-naproxen, but may undergo chiral inversion during wastewater treatment to form (*R*)-naproxen [9]

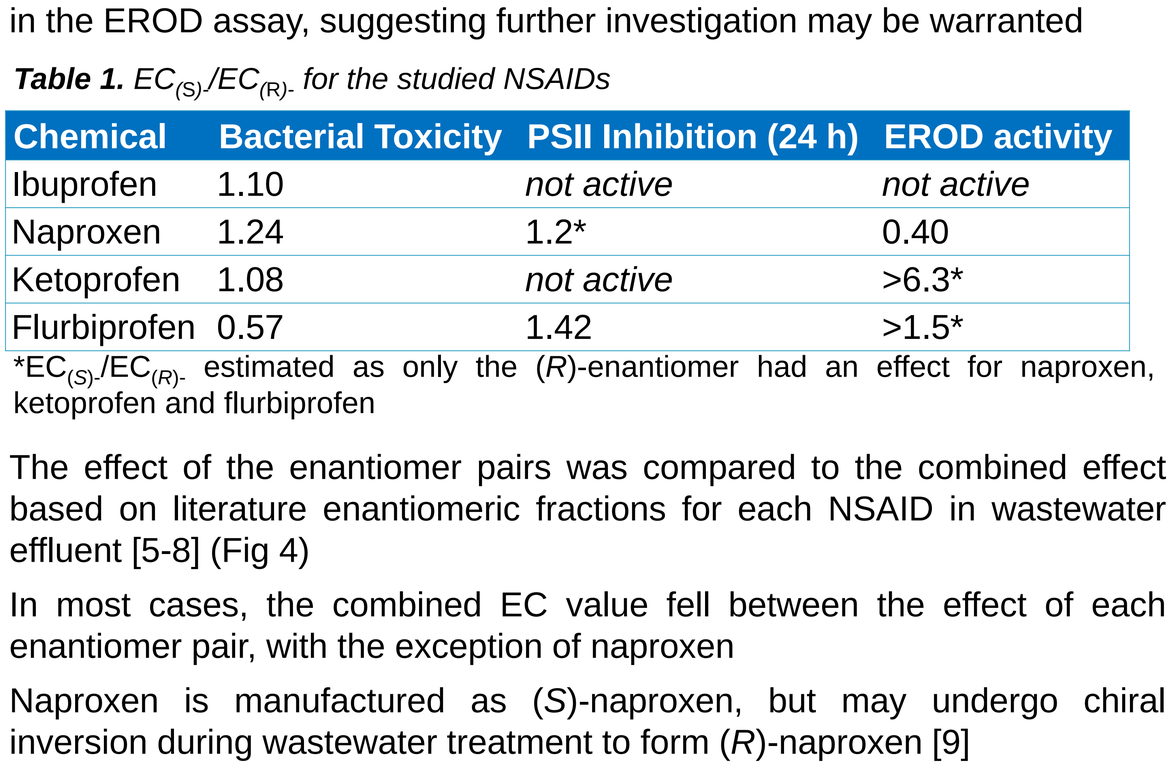


***Fig 4.*** *Combined EC values (solid bar) for the studied NSAIDs in wastewater effluent for assays indicative of bacterial toxicity, PSII inhibition (24 h) and EROD activity using enantiomeric fractions available in the literature. The EC value for the (S)-enantiomer is indicated by the S symbol, while the (R)-enantiomer is indicated by the R symbol*

**Conclusions:** While differences in effect between enantiomer pairs were observed, the differences in all assays was less than an order of magnitude. This suggests that enantiospecific effect data will not have a major impact on ecological risk assessment of NSAIDs, at least for the studied test systems

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**References:** [1] van de Merwe, Leusch (2015) *Environ. Sci.: Process. Impacts* 17: 947; [2] Repetto *et al.* (2008) *Nat. Protoc.* 3: 1125; [3] Escher *et al.* (2008) *J. Environ. Monit.* 10: 612; [4] Thibaut, Porte (2008) *Toxicol In Vitro* 22: 1128; [5] Matamoros *et al*. (2009) *Chemosphere* 75: 200; [6] Hashim, Khan (2011) *J. Chromatogr. A* 1218: 4746; [7] Khan *et al*. (2014) *Chirality* 26: 739; [8] Yuan *et al.* (2018) *Anal. Methods* 10: 4404; [9] Hashim *et al.* (2011) *Water Res*. 45: 6249