Environment International 136 (2020) 105464

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

# Current understanding of potential ecological risks of retinoic acids and their metabolites in aquatic environments

Katie Wan Yee Yeung[a], Guang-Jie Zhou[a,*], Klára Hilscherová[b], John P. Giesy[c,d], Kenneth Mei Yee Leung[a,e,*]

[a] The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China
[b] RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, Pavilion A29, 625 00 Brno, Czech Republic
[c] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[d] Department of Environmental Science, Baylor University, Waco, TX, United States
[e] State Key Laboratory of Marine Pollution (City University of Hong Kong), Tat Chee Avenue, Kowloon, Hong Kong, China

ARTICLE INFO

Handling Editor: Adrian Covaci

*Keywords:*
Retinoids
Sewage
Cyanobacteria
Aquatic environments
Ecological risks
Marine

ABSTRACT

In animals, retinoic acids (RAs), one of the main derivatives of vitamin A, are crucial for a variety of physiological processes. RAs, including all-*trans*-RA, 9-*cis*-RA, 13-*cis*-RA, and their corresponding metabolites (i.e., all-*trans*-4-oxo-RA, 9-*cis*-4-oxo-RA and 13-*cis*-4-oxo-RA) can be excreted through urination from humans and animals. Sewage treatment plants (STPs) are a significant source of RAs and 4-oxo-RAs into aquatic environments. RAs and 4-oxo-RAs can be identified and quantified by use of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). RAs and 4-oxo-RAs have been reported in various environmental matrices including rivers, lakes, reservoirs and coastal marine environments as well as in sewage effluents discharged from STPs. Greater concentrations of RAs and 4-oxo-RAs have been observed during blooms of cyanobacteria and microalgae, suggesting that cyanobacteria and microalgae are natural sources of RAs and 4-oxo-RAs in aquatic environments. These potential sources of RAs and 4-oxo-RAs raise concerns about their concentrations and risks in aquatic environments because excessive intake of these chemicals can result in abnormal morphological development in animals. Teratogenic effects were observed in amphibians, fish embryos, gastropods, mammals and birds when exposed to RAs. This review summarizes sources, concentrations, adverse effects and ecological risks of RAs and 4-oxo-RAs in aquatic environments. An interim, predicted no-effect concentration (PNEC) of RAs (in terms of at-RA) for freshwater environments was determined to be 3.93 ng/L at-RA equivalents. Based on limited data on concentrations of RAs in freshwater ecosystems, their hazard quotients were found to range from zero to 16.41, depending on the environmental conditions of receiving waters. Ecological risks of RAs in marine environments are yet to be explored due to the paucity of data related to both their concentrations in marine environment and toxic potencies to marine species. This review updates current knowledge of RAs and 4-oxo-RAs in aquatic environments and calls for more studies on their concentrations and fate in aquatic environments, especially estuarine and coastal marine environments with a view to enabling a comprehensive assessment of their ecological risks around the globe.

## 1. Introduction

Retinoids are a class of compounds consisting of four isoprenoid units (i.e., double bonds) joined in a head-to-tail manner (IUPAC–IUB, 1983). They play crucial roles in various biological processes in animals, including embryonic development, growth, reproduction, vision, homeostasis and regeneration of tissues and organs, cell differentiation and immune response (Barua and Furr, 1998; Collins and Mao, 1999; Ross et al., 2000). For animals, however, retinoids must be obtained from dietary sources of beta-carotene, such as fruits and vegetables (Gesto et al., 2012b; Novák et al., 2008). Beta-carotene is then converted to retinol (vitamin A) during digestion and then oxidized to retinal (Novák et al., 2008), which is subsequently irreversibly converted to retinoic acids (RAs) by aldehyde dehydrogenases (ALDHs) (Fig. 1). RAs can be further metabolized to various oxidative metabolites (4-oxo-RAs).

RAs belong among the main biologically active forms of retinoids, including retinal, RAs and 4-oxo-RAs. Due to the presence of conjugated

* Corresponding authors at: School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China.
*E-mail addresses:* zhougj01@gmail.com (G.-J. Zhou), kmyleung@hku.hk (K.M.Y. Leung).

https://doi.org/10.1016/j.envint.2020.105464
Received 30 September 2019; Received in revised form 13 December 2019; Accepted 2 January 2020
Available online 08 January 2020

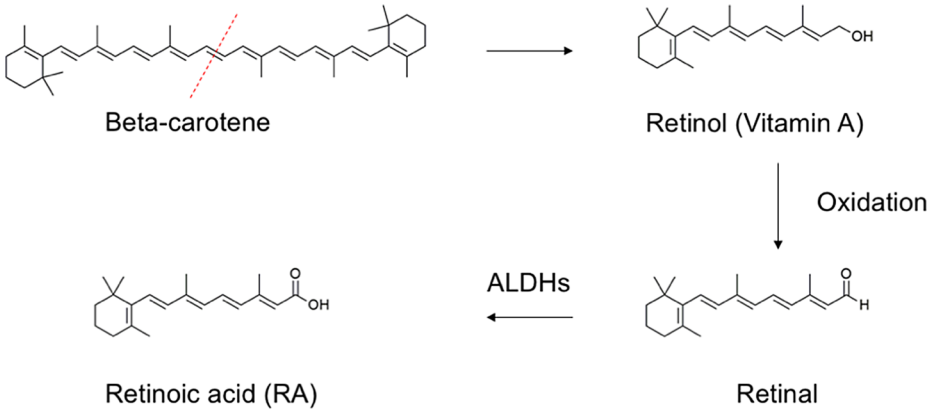


**Fig. 1.** Simplified metabolic pathway from beta-carotene in fruits and vegetables to retinoic acids in animal body.

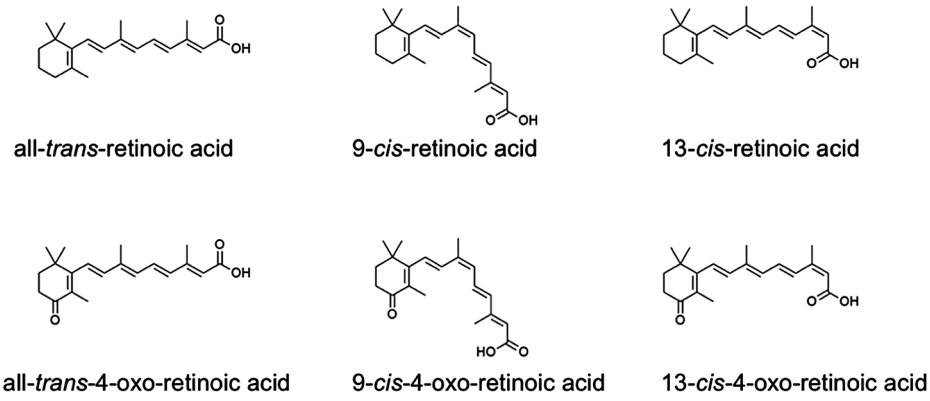


**Fig. 2.** Structures of retinoic acids and their metabolites discussed in this paper.

double bonds in RAs, they are relatively unstable and thus exhibit different forms and isomers, including all-*trans*-RA (at-RA), 9-*cis*-RA (9c-RA) and 13-*cis*-RA (13c-RA), and also isomers of their corresponding derivatives 4-oxo-RAs, including all-*trans*-4-oxo-RA (at-4-oxo-RA), 9-*cis*-4-oxo-RA (9c-4-oxo-RA) and 13-*cis*-4-oxo-RA (13c-4-oxo-RA) (Fig. 2) (Barua and Furr, 1998). These six compounds: at-RA, 9c-RA, 13c-RA, at-4-oxo-RA, 9c-4-oxo-RA and 13c-4-oxo-RA, will be the focus of this review paper. They are all naturally occurring retinoids and each of them can modulate the expression of genes through retinoid signalling pathways, which are mediated by two classes of nuclear receptor families, the retinoid X receptor (RXR) and retinoic acid receptor (RAR) (Gardiner et al., 2003; Novák et al., 2008). Both RXR and RAR are responsible for regulating cell growth and differentiation in various organisms. At-RA and 13c-RA and their corresponding metabolites (i.e., at-4-oxo-RA and 13c-4-oxo-RA) bind to RAR while 9c-RA binds to both RAR and RXR with stronger affinity to RXR (Balmer and Blomhoff, 2002; Novák et al., 2008). RARs are activated by at-RA, 9c-RA, 13c-RA, at-4-oxo-RA and 13c-4-oxo-RA, while RXRs are activated by 9c-RA only (Fig. 3; Collins and Mao, 1999; Gardiner et al., 2003; Novák et al., 2008). The activation of RXRs by at-RA is unclear. In order to bind with DNA and activate the transcription of RAR target genes, RAR can only be active in the presence of RXR by forming heterodimers, while RXR can be active in the absence of ligands (Gardiner et al., 2003).

Since RAs play crucial roles in biological processes, either insufficient or excessive amounts of RAs can result in teratogenic effects on animals. Such teratogenic effects have been widely observed in amphibians, fishes and gastropods in aquatic environments (Gardiner et al., 2003; Horiguchi et al., 2008; Jonas et al., 2014, 2015; Pipal et al., 2020; Zhu et al., 2014). In recent years, RAR agonistic activity has been detected in effluents discharged from sewage treatment plants (STPs) and/or in their receiving rivers in Australia (Allinson et al., 2011), China (Zhen et al., 2009; Wu et al., 2010), Germany (Völker et al., 2016) as well as in Japan (Inoue et al., 2010, 2011, 2013; Sawada et al., 2012). The major RAR agonists in sewage were at-RA and 13c-RA, and their oxidative metabolites at-4-oxo-RA and 13c-4-oxo-RA (Zhen et al., 2009; Sawada et al., 2012). A recent study demonstrated that RAs and 4-oxo-RAs were present in wastewater and sludge from STPs of Hong Kong, and their adjacent receiving seawaters (Zhou et al., 2019). Several studies have also documented the presence of RAs and their derivatives in water bodies during blooms of cyanobacteria (Wu et al., 2012; Javůrek et al., 2015; Sehnal et al., 2019). However, there is insufficient information on ecological risks posed by RAs in aquatic environments. Based on currently available information about RAs, the objectives of this review were to: (1) identify potential sources of RAs and 4-oxo-RAs contributed to aquatic environments; (2) discuss analytical methods used to identify and quantify RAs and 4-oxo-RAs in surface waters, sewage, sludge, cyanobacteria and other biota; (3) summarize concentrations of RAs and 4-oxo-RAs in receiving water bodies around the world; (4) recapitulate toxic potencies of RAs and 4-oxo-RAs to various species; and (5) evaluate ecological risks of RAs and 4-oxo-RAs, based on the detected concentrations present in aquatic environments and their toxic effects to aquatic organisms.

## 2. Sources of RAs and 4-oxo-RAs

RAs and 4-oxo-RAs have been detected in various environmental matrices including effluent and sludge from STPs (Zhen et al., 2009; Wu et al., 2010; Sawada et al., 2012; Inoue et al., 2013; Zhou et al., 2019), adjacent receiving seawaters (Zhou et al., 2019), rivers (Zhen et al., 2009; Wu et al., 2010), lakes and reservoirs especially during cyanobacterial blooms (Wu et al., 2012; Javůrek et al., 2015; Sehnal et al., 2019). Retinoids in aquatic environments can come from both natural and artificial sources.

Beta-carotene is abundant in fruits and vegetables, which are one of

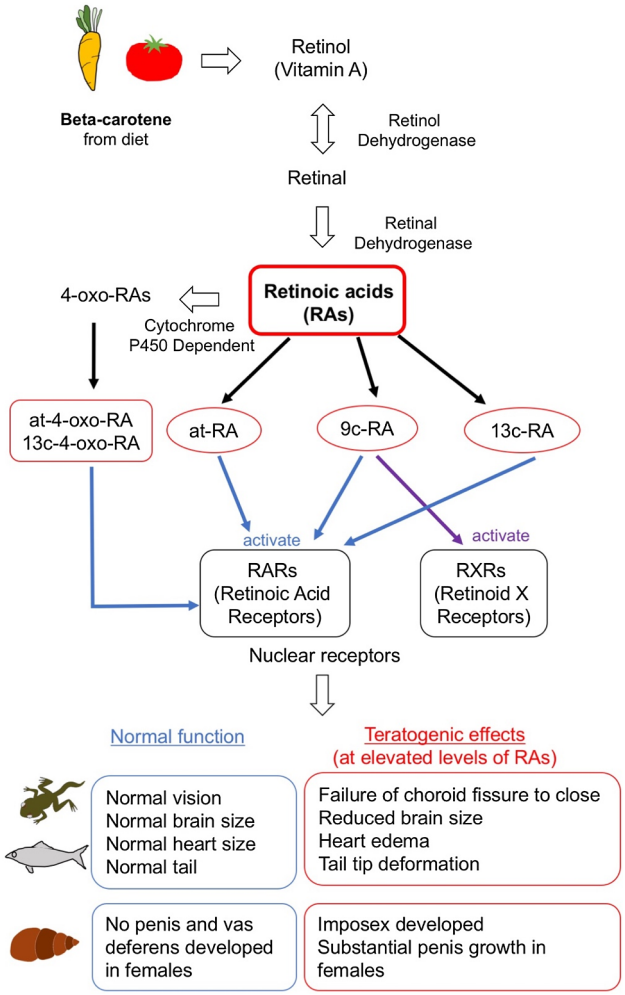


**Fig. 3.** Simplified pathway of retinoic acids from beta-carotene in diet to retinoic acids in aquatic animals' body, and the development of teratogenic effects at elevated levels in various groups of aquatic animals including fish, amphibians and gastropods (Collins and Mao, 1999; Gardiner et al., 2003; Novák et al., 2008).

the major ingredients in diets of humans and animals (Maiani et al., 2009). After ingestion, beta-carotene can be transformed (Fig. 1). RAs and 4-oxo-RAs are normally present in plasma of humans and animals and under normal physiological conditions, they are excreted in urine (Lambert and De Leenheer, 1985). Among the six compounds of interest, 13c-RA is the dominant form of RA in urine whereas at-RA and 9c-RA are present at relatively lesser concentrations (Lambert and De Leenheer, 1985). Due to their incomplete treatment, RAs and 4-oxo-RAs can enter aquatic environments through effluent discharges of STPs. Among RAs and 4-oxo-RAs, 9c-RA and 9c-4-oxo-RA are, in general, less likely to be detected than other RAs and their metabolites. This is probably due to 9c-RA being a less dominant form in urine under normal physiological conditions, while 13c-RA is the most favourable form to be excreted (Lambert and De Leenheer, 1985).

Apart from domestic sewage, concentrations of RAs and 4-oxo-RAs detected during blooms of cyanobacteria were greater than those detected in the absence of blooms in any aquatic systems, including coastal seawater, freshwater and effluent from sewage treatment plants, where the maximum concentration of RAs was 421 ng/L (sum of intracellular and extracellular content) (Wu et al., 2012). RAs can be transformed from their precursors (such as retinal and beta-carotene) which are produced by cyanobacteria and/or microalgae (Wu et al., 2013). For instance, RAs can be transformed from retinal by cyanobacterial aldehyde dehydrogenases (ALDH) (Miles et al., 2019; Fig. 1). This illustrates the fact that cyanobacteria and microalgae are possible sources of RAs and 4-oxo-RAs in aquatic environments.

Besides, retinoids are commonly used in drugs and food industries since various isomers of RAs exhibit various functions (Lucek and Colburn, 1985). For instance, 30 mg 9c-RA/day effectively treated *Pityriasis rubra pilaris* (Amann et al., 2015); and a maximum of 1.2 mg 13c-RA/kg/day is used to cure cystic acne (Farrell et al., 1980; Peck et al., 1982). More importantly, at-RA is effective at treating acute promyelocytic leukaemia (Huang et al., 1988; Wang and Chen, 2008). Application of drugs is another potential source of RAs and 4-oxo-RAs in aquatic environments.

## 3. Analytical methods of RAs and 4-oxo-RAs

Methods for extraction, identification and quantification of RAs and 4-oxo-RAs in surface waters, sewage, sludge and biota samples have been developed. Because they are easily isomerized and metabolized, extraction is a crucial step in quantifying RAs. In general, water samples, including river water, seawater and sewage, are filtered with filter papers prior to solid phase extraction (SPE) (Zhen et al., 2009; Inoue et al., 2013; Zhou et al., 2019). The SPE cartridge is normally preconditioned with ethyl acetate, methanol and water although there is no agreement on the most appropriate solvent used for preconditioning. Because matrix components can be present, a clean-up procedure is usually required, with the use of silica gel as adsorbents, after SPE (Wu et al., 2010; Zhou et al., 2019). For sludge, freeze-dried samples are extracted with ethyl acetate, which is then purified following clean-up procedures (Zhou et al., 2019). Internal standards, deuterated at-RA and acitretin for RAs and 4-oxo-RAs, respectively, were usually added to determine the recoveries of selected target compounds. Recoveries of RAs and 4-oxo-RAs in influent, effluent, sludge, river water and seawater, were 53%–160%, 52%–73%, 70%–158%, 53%–61% and 52%–74%, respectively (Wu et al., 2010; Zhou et al., 2019).

Procedures for extraction of RAs and 4-oxo-RAs from biota are similar to those used for extraction from water, except that there are additional steps prior to extraction using SPE. Tissue samples of gastropods and fish are homogenized, mixed with methanol and centrifuged. The supernatants obtained after homogenate centrifugation are extracted using SPE (Gesto et al., 2012a, 2012b). For cyanobacteria and algae, cells and exudates are separated by centrifugation and/or filtration. Supernatants (i.e., exudates) are then extracted by use of SPE, while filtered algal or cyanobacterial cells are extracted by sonication and centrifugation in the presence of solvent (Wu et al., 2012; Jonas et al., 2014). Similar clean-up procedures with the use of silica gel are also applied for biota samples to remove matrix components, such as lipids and proteins.

Due to the presence of conjugated double bonds, RAs are easily isomerized and oxidized, and are sensitive to effects of heat, light and oxygen (van Breemen et al., 1998). Therefore, extraction should comply with strict procedures (Zhou et al., 2019). For sample collection, amber bottles should be used and samples should be extracted as soon as possible (usually within six hours). If possible, extractions should be conducted in the dark, while all cartridges used for SPE and clean-up should be wrapped in aluminium foil. Extracts should be dried using nitrogen gas instead of air to avoid possible oxidation of chemicals and they should be stored at −18 °C until identification and quantification by LC-MS/MS.

An analytical technique with specificity and sufficient limit of detection to be environmentally relevant and excellent power to resolve individual compounds is needed for identification and quantification of RAs and 4-oxo-RAs. Since liquid chromatography (LC) has a mild separation conditions such as lower column temperature and less complicated sample preparation procedures compared to gas chromatography (GC), LC is commonly employed for separation of RAs and 4-oxo-RAs. Higher temperatures employed in GC (usually 230 °C) might cause isomerization of RAs and destroy geometric isomers during

separation (van Breemen et al., 1998; Wang et al., 2001). Time and cost can also be saved using LC since derivatization is not needed for separation. The major challenge using LC is that the isomers of RAs and 4-oxo-RAs are difficult to be isolated in LC columns, even this issue can be addressed through the addition of acetic acids (for RAs) and ammonium acetate (for 4-oxo-RAs) in the mobile phases (Wu et al. 2010; Zhou et al., 2019). Since RAs have strong molar absorptivity at both UV and visible wavelengths with the presence of conjugated double bonds, LC is conventionally coupled with ultraviolet–visible spectroscopy (UV–Vis) for detection of RAs (van Breemen et al., 1998). Nevertheless, UV–Vis usually has relatively poor limits of quantification. A complementary identification technique of mass spectrometry (MS) is, therefore, a more suitable detector for identification and quantification of RAs (van Breemen et al., 1998). The use of MS has the added advantage of providing accurate mass and fragmentation patterns to retention time to make it possible to more accurately identify individual compounds.

With the improvement of technology, tandem mass spectrometer (MS/MS) using soft ionization methods such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) is currently used for quantification of RAs (Gundersen et al., 2007; Zhou et al., 2019). MS/MS can increase the throughput with high resolution and it has better selectivity with lower background levels of RAs (Gundersen et al., 2007; Wu et al., 2010). Among ionization methods, APCI, in either positive or negative mode, generates stronger signal, provides better linearity at high concentration and possesses wider dynamic range when compared with ESI (van Breemen et al., 1998; Wang et al., 2001). Although ESI is more susceptible to matrix effects than APCI that would suppress signal, the use of surrogate standards can achieve method recoveries between 51% and 81.7% (Wu et al. 2010). Also, it has recently been shown that ESI provides better sensitivities for retinoids, lower limit of detection (LOD) and limit of quantitation (LOQ) than APCI that is sufficient for environmental analysis (Wu et al., 2013). ESI is, therefore, more commonly used as the ionization mode for quantification at present. Furthermore, to prevent peak distortion and to achieve better separation, elution conditions of mobile phases are usually acidic for RAs or alkaline for 4-oxo-RAs (Wang et al., 2001; Wu et al., 2010; Zhou et al., 2019). Formic and acetic acids are commonly used for acidification, and ammonium acetate is used for alkalization. The LOD and LOQ for quantification are usually established as the least concentration of analyte in samples that corresponds to signal-to-noise (S/N) ratio of 3 and 10, respectively (Gundersen et al., 2007; Wu et al., 2010; Zhou et al., 2019). LODs and LOQs differ among identification and quantification methods.

## 4. Concentrations of RAs and 4-oxo-RAs in aquatic environments

Concentrations of RAs and 4-oxo-RAs have been measured in various environmental matrices including seawater, rivers, reservoirs, lakes and ponds, effluent and sludge from STPs (Table 1). Since RAs and 4-oxo-RAs are normally excreted through urination from animals, this matches with their continuing detection at concentrations up to 100 ng/L in influents of STPs (Zhen et al., 2009; Wu et al., 2010; Sawada et al., 2012; Inoue et al., 2013; Zhou et al., 2019). Concentrations of RAs in effluents were consistently less than those in influents (Zhen et al., 2009; Wu et al., 2010; Zhou et al., 2019). The greatest concentration of single RAs or 4-oxo-RAs detected in influents of STPs was 104.9 ng 13c-4-oxo-RA/L (Inoue et al., 2013), which is more than 10-fold greater than the greatest concentration detected in effluents, surrounding seawaters or rivers near discharges of STPs with 2.4 ng 13c-4-oxo-RA/L observed (Zhou et al., 2019). STPs might, therefore, play a role in removing RAs and 4-oxo-RAs during treatment. To better understand which treatment process is the most efficient for removing RAs, further studies on removal efficiencies of various types of sewage treatment are warranted.

Retinoids can be produced and released as extracellular products during blooms of cyanobacteria, which are dominated by species belonging to various genera, including *Microcystis, Dolichospermum, Planktothrix, Woronichinia* and *Pseudanabaena* (Sehnal et al., 2019). Retinoids were detected in all 17 water bodies during blooms of cyanobacteria, with the sum of analyzed extracellular and intracellular RAs and 4-oxo-RA up to 109 ng/L and 1,441 ng/g dw in biomass, respectively (Sehnal et al., 2019). Moreover, during blooms of cyanobacteria dominated by *Microcystis aeruginosa* in the Tai Lake (Ch:*Taihu*), maximum values for intracellular and extracellular RAs and 4-oxo-RAs were 420 ng/L and 19.8 ng/L, respectively (Wu et al., 2012). Other studies also documented that freshwater cyanobacteria can, especially during blooms, produce RAs and their metabolites (Kaya et al., 2011; Javůrek et al., 2015; Sychrová et al., 2016; Priebojová et al., 2018). Results of a few studies have found that total concentrations of intracellular and extracellular RAs and 4-oxo-RAs for a wide spectrum of laboratory cultured cyanobacteria and microalgae were as much as 1,919 ng/g dry mass (dm) and 2,390 ng/L, respectively (Wu et al., 2012; Sychrová et al., 2016; Priebojová et al., 2018). In freshwater, algal species originated from various algal groups including green algae, diatoms and euglena also have the ability to produce RAs even though the concentrations were less than those from some species of cyanobacteria (Wu et al., 2012). Cyanobacteria are known to produce a variety of substances during their growth, while some of them, such as microcystins and retinoids, can cause adverse effects on aquatic organisms (Wu et al., 2012; Paerl and Otten, 2013). However, data on concentrations of RAs in marine environments are lacking (Table 1). Until now, there have been only two studies of RAs in marine environments (Wu et al., 2010; Zhou et al., 2019). It is, therefore, necessary to conduct more surveys in marine environments to detect RAs, especially in coastal cities that are densely populated and urbanized.

## 5. Toxic effects of RAs and 4-oxo-RAs, and possible mechanisms

Toxicities of RAs and 4-oxo-RAs have been determined for various animal species (Table 2). Although these species respond differently to RAs, most of them showed abnormal morphological development when exposed to elevated concentrations (Table 2; Fig. 3). As amphibians are sensitive to environmental changes and they breathe through the skin partially, many studies have used amphibians to study the toxic effects of RAs. Malformations, including, but not limited to, reduced brain size, abnormal eyes, bent tails and abnormal hindleg formation, were consistently observed in amphibian embryos at the range of median effect concentration ($EC_{50}$) of 7.57–23.2 μg at-RA/L (Table 2). In embryos of fishes, tail tip deformation, brain deformities and heart edema were commonly observed with $EC_{50}$ of 0.10–1.15 μg at-RA/L (Table 2). For mammals and birds, rat and chicken embryos (i.e., eggs) were usually used for determining toxic potencies of RAs (Summerbell, 1983; Cicurel and Schmid, 1988; Ritchie et al., 2003). Malformations, such as growth retardation of mammals and birds, were observed at greater concentrations compared to thresholds for effects in amphibians and fishes. Studies on *Xenopus laevis* embryos (Franco et al., 1999), newts *Notophthalmus viridescens* (Stark et al., 1998) and zebrafish embryos (Hoffman et al., 2002) suggested that these malformations may be induced due to the involvement of the sonic hedgehog (shh) gene which regulates different development processes such as limb development and pectoral fin bud expression.

Although several studies were conducted to determine toxic effects of concentrations of RAs on freshwater species, there were only three studies on marine species, i.e., the olive flounder *Paralichthys olivaceus* and the rock shell *Reishia (=Thais) clavigera* (Haga et al., 2002; Nishikawa et al., 2004; Horiguchi et al., 2008). Thus, there is still a lack of information on toxic effects and mechanisms of RAs on marine species. Previous laboratory studies indicated that 9c-RA triggered development of imposex in females of *R. clavigera* (Nishikawa et al., 2004; Horiguchi et al., 2008). Results of a recent study suggested that RAs originating from STP effluents might contribute to development of imposex in females of *R. clavigera* in the marine environment of Hong

**Table 1**
Concentrations of retinoic acids (RAs) and their metabolites (4-oxo-RAs) in various environmental matrices including seawater, rivers and lakes, sewage treatment plants (both sewage and sludge) and during cyanobacterial blooms.

| Country | Location | at-RA (ng/L) | 9c-RA (ng/L) | 13c-RA (ng/L) | at-4-oxo-RA (ng/L) | 9c-4-oxo-RA (ng/L) | 13c-4-oxo-RA (ng/L) | References |
|---|---|---|---|---|---|---|---|---|
| **Seawater** | | | | | | | | |
| China | Liaodong Bay | < 0.02 | < 0.04 | < 0.03 | < 0.02 | < 0.05 | < 0.06 | Wu et al. (2010) |
| | Hong Kong–Coastal waters | ND – 0.08 | ND | ND– 0.10 | 0.81–1.33 | ND – 0.78 | 0.99–2.41 | Zhou et al. (2019) |
| **Rivers** | | | | | | | | |
| China | Rivers adjacent to Liaodong Bay | 0.05–1.23 | < 0.06 | < 0.03–0.41 | < 0.02–1.00 | < 0.10 | < 0.06–0.81 | Wu et al. (2010) |
| | Tonghui river and Qing river | NA | NA | NA | < 0.2–1.0 (July 2006)<br>< 0.2–1.8 (January 2007) | NA | < 0.4–1.6 (July 2006)<br>< 0.4–1.5 (January 2007) | Zhen et al. (2009) |
| **Sewage Treatment Facilities** | | | | | | | | |
| China | Panjin – Influent | 2.41 | < 0.35 | 0.74 | 17.01 | 2.79 | 12.45 | Wu et al. (2010) |
| | Panjin – Effluent | 0.06 | < 0.05 | 0.06 | < 0.04 | < 0.04 | < 0.09 | Wu et al. (2010) |
| | Panjin – Lagoon from duck farm | 0.97 | < 0.35 | 0.36 | 1.64 | < 0.11 | 1.23 | Wu et al. (2010) |
| | Beijing - Influent | NA | NA | NA | 4.7–10.4 | NA | 2.3–7.1 | Zhen et al. (2009) |
| | Beijing - Effluent | NA | NA | NA | < 0.02–0.9 | NA | < 0.4–1.1 | Zhen et al. (2009) |
| | Hong Kong – Influent | 1.58 | ND | 2.00–4.11 | 3.51–22.47 | ND | ND–3.22 | Zhou et al. (2019) |
| | Hong Kong – Effluent | 0.79 | ND | 1.00 | 0.45–5.67 | ND | 0.60–1.64 | Zhou et al. (2019) |
| | Hong Kong – Sludge | 4.8–17.0 (ng/g dw) | ND | 9.0–11.0 (ng/g dw) | ND | ND | ND | Zhou et al. (2019) |
| Japan | Osaka – Influent | < 1.0–5.0 | NA | < 5.0 | < 0.5–5.3 | NA | 1.7–70.2 | Sawada et al. (2012) |
| | Osaka – Effluent | < 0.5 | NA | < 2.5 | < 0.25 | NA | < 0.5–0.6 | Sawada et al. (2012) |
| | Osaka – Influent | < 4.3–21.5 (March)<br>< 4.3–17.3 (July) | NA | < 13.6 (March)<br>< 13.6 (July) | < 0.65–6.9 (March)<br>< 0.65–1.5 (July) | NA | 2.3–94.3 (March)<br>9.9–104.9 (July) | Inoue et al. (2013) |
| | Osaka – Effluent | < 1.4 (March)<br>< 1.4–11.5 (July) | NA | < 4.5 (March & July) | < 0.41 (March & July) | NA | < 0.73–0.83 (March)<br>< 0.73 (July) | Inoue et al. (2013) |
| **Reservoirs, lakes and ponds** | | | | | | | | |
| Czech Republic | South Moravian region – Intracellular | < 1.5–340 (ng/g dm) | < 1.5–84 (ng/g dm) | NA | NA | NA | NA | Javůrek et al. (2015) |
| | South Moravian region – Extracellular | < 0.15–19 | < 0.15–2.2 | NA | NA | NA | NA | Javůrek et al. (2015) |
| | South Bohemia and south Moravia – Intracellular | < 3–353 (n g/g dm) | < 1–46.5 (ng/g dm) | | < 1–1088 (ng/g dm) | NA | NA | Sehnal et al. (2019) |
| | South Bohemia and south Moravia – Extracellular | < 0.25–27.5 | < 0.03–1.73 | | < 0.08–79.9 | NA | NA | Sehnal et al. (2019) |
| China | Taihu Lake – Intracellular | < 0.3–220 | < 0.8 | < 0.3–120 | 0.1–160 | < 0.1–21 | < 0.1–21 | Wu et al. (2012) |
| | Taihu Lake – Extracellular | < 0.2–5.9 | < 0.4–3.2 | < 0.2–5.8 | < 0.06–6.0 | < 0.1–1.0 | < 0.1 | Wu et al. (2012) |

at-RA, all-*trans*-retinoic acid; 9c-RA, 9-*cis*-retinoic acid; 13c-RA, 13-*cis*-retinoic acid; at-4-oxo-RA, all-*trans*-4-oxo-retinoic acid; 9c-4-oxo-RA, 9-*cis*-4-oxo-retinoic acid; 13c-4-oxo-RA, 13-*cis*-4-oxo-retinoic acid
NA, not analyzed; ND, not detected.

**Table 2**
Toxicity data of retinoic acids (RAs) and 4-oxo-RAs towards different animal species.

| Taxa | Species | Duration of exposure (h) | $LC_{50}$ (μg/L) | $EC_{50}$ for malformations (μg/L) | LOEC for malformations (μg/L) | References |
|---|---|---|---|---|---|---|
| Amphibian | *Rana clamitans* (embryo) | 24 | at-RA = 20.9 | NA | NA | Degitz et al. (2000) |
| | *Rana septentrionalis* (embryo) | 24 | at-RA = 16.0 | at-RA = 13.5–23.2 | NA | Degitz et al. (2000) |
| | *Xenopus laevis* (embryo) | 1 | NA | NA | at-RA = 300 | Franco et al. (1999) |
| | *Xenopus laevis* (embryo) | 24 | at-RA = 8.8 | at-RA = 7.57–11.5 | NA | Degitz et al. (2000) |
| | *Xenopus laevis* (embryo) | 96 | at-RA = > 20 | at-RA = 11.9 | at-RA = 5–20 | Smutná et al. (2017) |
| | *Xenopus laevis* (tadpoles) | 48 or 120 | NA | NA | at-RA = 10 μg/g | Alsop et al. (2004) |
| | *Xenopus tropicalis* (embryo) | 48 | NA | NA | at-RA = 5<br>9c-RA = 0.5 | Hu et al. (2015) |
| | *Xenopus tropicalis* (embryo) | 48 | NA | NA | 9c-RA = 2.5 | Zhu et al. (2014) |
| | *Xenopus tropicalis* (embryo) | 72 | NA | NA | at-RA = 1<br>9c-RA = 1 | Yu et al. (2011) |
| Fish | *Danio rerio* (embryo) | 120 | NA | NA | at-RA = 0.9<br>9c-RA = 3<br>13c-RA = 3<br>at-4-oxo-RA = 3<br>13c-4-oxo-RA = 30 | Herrmann (1995) |
| | *Danio rerio* (embryo) | 10, 16, 24, 36, 48 | NA | NA | at-RA = 30 | Hoffman et al. (2002) |
| | *Danio rerio* (embryo) | 96 | at-RA = 108 | at-RA = 1.1–20.8 | at-RA = 1.3–36.0 | Jonas et al. (2014) |
| | *Danio rerio* (embryo) | 120 | at-RA = 5.2 | at-RA = 0.7 | NA | Wang et al. (2014) |
| | *Danio rerio* (embryo) | 24, 48, 72, 144 | at-RA = 7.74–483.7 | at-RA = 0.1–28 | NA | Selderslaghs et al. (2009) |
| | *Danio rerio* (embryo) | 24, 48, 72, 144 | at-RA = 40.2–2121.1 | at-RA = 0.42 –233.7 | NA | Selderslaghs et al. (2012) |
| | *Danio rerio* (embryo) | 120 | at-RA = 5.47 | at-RA = 1.15 | at-RA = 0.8 | Pipal et al. (2020) |
| | *Paralichthys olivaceus* | 96 | NA | NA | at-RA = 7.5<br>9c-RA = 7.5<br>13c-RA = 7.5 | Haga et al. (2002) |
| Invertebrate | *Pomacea canaliculata* | 15 days and 30 days | NA | NA | 9c-RA = 1 μg/g of drained body mass | Giraud-Billoud and Castro-Vazquez (2019) |
| | *Reishia (=Thais) clavigera* | 1 month | NA | NA | 9c-RA = 1 μg/g of drained body mass | Horiguchi et al. (2008)<br>Nishikawa et al. (2004) |
| Mammal | Rat embryo *in vitro* | 48 | NA | Only $EC_{30}$ is available:<br>at-RA = 30<br>13c-RA = 3000 | NA | Cicurel and Schmid (1988) |
| | Sprague-Dawley Rat embryo *in vitro* | 46 | NA | NA | at-RA = 49.8<br>9c-RA = 49.8<br>at-4-oxo-RA = 9.9 | Ritchie et al. (2003) |
| Bird | Chicken eggs | 10 days | NA | NA | at-RA = $2.5 \times 10^5$ | Summerbell (1983) |

at-RA, all-*trans*-retinoic acid; 9c-RA, 9-*cis*-retinoic acid; 13c-RA, 13-*cis*-retinoic acid; at-4-oxo-RA, all-*trans*-4-oxo-retinoic acid; 13c-4-oxo-RA, 13-*cis*-4-oxo-retinoic acid.
$LC_{50}$, median lethal concentrations; $EC_{50}$, median effect concentrations; LOEC, lowest observed effect concentration
NA, not applicable.

Kong (Zhou et al., 2019). Although RXR/RAR mediated pathway of RAs might be similar to that of other environmental pollutants such as tributyltin and triphenyltin, the mechanism of RA-induced imposex in gastropods *R. clavigera* is very complex and it is not clearly known yet. Molecular mechanisms underlying RA signaling functions that lead to malformations are not conserved across all animal species, and are different between vertebrates and invertebrates. Furthermore, the physiological role of RA signaling in invertebrates is still largely unknown. Our knowledge on biological responses and effects of RAs on different taxonomic groups are still limited.

Apart from the insufficient information on toxic effects of RAs on marine species, previous studies usually focus on the toxic effect on RAs rather than 4-oxo-RAs. However, 4-oxo-RAs might sometimes exhibit greater bioactivities than RAs because 4-oxo-RAs are the oxidized form of RAs with better stability. The toxic effects and mechanisms of 4-oxo-RAs on aquatic species are warranted to be further investigated.

## 6. Ecological risk assessment

All-*trans* retinoic acid equivalents (at-RA EQs) are usually used to express the total retinoid activity in samples with respect to at-RA standards (Jonas et al., 2014). Equivalency factors (RAEFs) for the at-RA were usually developed for each retinoid to an equivalent concentration of at-RA for characterizing and comparing the potency of samples. The calculation is based on the concept of relative potencies, which are expressed as the ratio of effective concentration of standard at-RA ($EC_x$ at-RA) and that of the sample (i.e., $EC_x$ sample) in a bio-detection system, mostly reporter gene assays detecting interaction of the samples with RAR (Villeneuve et al., 2000; Jonas et al., 2014; Javůrek et al., 2015; Sehnal et al., 2019). However, the slopes of the dose-response curves must be the same. Otherwise, the ratio of $EC_{20}$ values would be used to minimize the bias (Villeneuve et al., 2000). Thus, a point estimation, which represents a single point estimation along a wider range of relative potencies, would be used in the calculation (Villeneuve et al., 2000).

RAEFs were defined as the $EC_{50}$ of a retinoid that was estimated by determining RARα-mediated activity using different bio-detection models, such as yeast two hybrid model and mammalian transgenic cells, relative to that of at-RA such that the toxicities of different retinoids are comparable (Zhen et al., 2009; Wu et al., 2010; Priebojová et al., 2018; Sehnal et al., 2019). The at-RA EQs of samples were calculated by summing the concentration of each retinoid measured in the study multiplied by their respective RAEFs (Wu et al., 2010; Zhou et al., 2019).

Since different bio-detection models, such as yeast two hybrid model and mammalian transgenic cells, can produce different RAEFs and eventually different at-RA EQs, at-RA EQs derived from RAEFs should be obtained from the same bioassay that is then used for the

**Table 3**
Selected toxicity data of retinoid equivalent of all-*trans* retinoic acids (at-RA EQ) towards five freshwater species, which belong to two taxa, for constructing species sensitivity distribution (SSD). For studies having a range of $EC_{50}$ values, the minimum value of $EC_{50}$ was selected for calculation. For species involved in multiple studies, a geometric mean of $EC_{50}$s was calculated. Malformation/damage to affected organs/tissues was found at the respective effective concentration.

| Taxa | Species | $LC_{50}$, LOEC or $EC_{50}$ (μg/L) | $EC_{50}$ (μg/L at-RA EQ) | Geometric mean of $EC_{50}$ (μg/L) | Affected organs/tissues | References |
|---|---|---|---|---|---|---|
| Amphibian | *Rana clamitans* (embryo) | at-RA = 20.9^ | 20.9^ | 20.9 | Brain, eyes, notochord, tail | Degitz et al. (2000) |
| | *Rana septentrionalis* (embryo) | at-RA = 13.5 | 13.5 | 13.5 | Brain, eyes, notochord, tail | Degitz et al. (2000) |
| | *Xenopus laevis* (embryo) | at-RA = 7.57 | 7.57 | 9.49 | Brain, eyes, notochord, tail | Degitz et al. (2000) |
| | *Xenopus laevis* (embryo) | at-RA = 11.9 | 11.9 | | Eye, gut, tail | Smutná et al. (2017) |
| | *Xenopus tropicalis* (embryo) | at-RA = 5* | 5* | 1.23 | Brain, cement gland, craniofacial, eye, fin, gut, heart, notochord, pigmentation, somite, trunk, tail | Hu et al. (2015) |
| | *Xenopus tropicalis* (embryo) | 9c-RA = 2.5* | 0.375* | | Anus, brain, eyes, fin, notochord, proctodaeum, tail, yolk | Zhu et al. (2014) |
| | *Xenopus tropicalis* (embryo) | at-RA = 1* | 1* | | Brain, eyes, heart, notochord, pigmentation, tail | Yu et al. (2011) |
| Fish | *Danio rerio* (embryo) | at-RA = 0.9* | 0.9* | 1.00 | Brain, hatching, heart, tail, yolk sac | Herrmann (1995) |
| | *Danio rerio* (embryo) | at-RA = 30* | 30* | | Fin | Hoffman et al. (2002) |
| | *Danio rerio* (embryo) | at-RA = 1.1 | 1.1 | | Hatching, heart, length, spine, tail | Jonas et al. (2014) |
| | *Danio rerio* (embryo) | at-RA = 0.7 | 0.7 | | Pericardial, pigmentation, swim bladder, tail, yolk sac | Wang et al. (2014) |
| | *Danio rerio* (embryo) | at-RA = 0.1 | 0.1 | | Eyes, hatching, heart beat, otoliths, skeletal, swimming ability, tail | Selderslaghs et al. (2009) |
| | *Danio rerio* (embryo) | at-RA = 0.42 | 0.42 | | Eyes, hatching, heart beat, otoliths, skeletal, swimming ability, tail | Selderslaghs et al. (2012) |
| | *Danio rerio* (embryo) | at-RA = 1.15 | 1.15 | | Heart, lower jaw, spine, swim bladder, tail | Pipal et al. (2020) |

* LOEC was used as $EC_{50}$ was not available in the study.
^ $LC_{50}$ was used as neither $EC_{50}$ nor LOEC were available in the study.

assessment of total retinoid activity of mixtures in environmental matrices. However, bioassays have not always been used to quantify the integrated measure of at-RA EQs given that some studies did not have RAEFs or at-RA EQs. Even though not every study had conducted bioassays, the measurement of individual RAs and 4-oxo-RAs was usually conducted using LC-MS/MS. In this review, RAEFs used were thus based on previously reported results (Zhen et al., 2009; Wu et al., 2010). Both of those authors used yeast, two-hybrid assays with all target RAs and 4-oxo-RAs, including 9c-RA, 13c-RA, at-4-oxo-RA, 9c-4-oxo-RA and 13c-4-oxo-RA. Values of individual RAs were multiplied by corresponding RAEFs, for 9c-RA, 13c-RA, at-4-oxo-RA, 9c-4-oxo-RA and 13c-4-oxo-RA, which were 0.15, 0.04, 3.87, 0.46 and 0.46, respectively.

Since not all studies used at-RA as the test chemicals, RAEFs were applied to convert the test RA to at-RA equivalents. Due to the limited data available on toxic potencies, we could only obtain acute *in vivo* toxicity data of at-RA for a total of five freshwater species, which belong to two taxa (four amphibians and one fish), from peer-reviewed literature (Table 3). Geometric means were applied when multiple toxicity data were available for the same species (Wheeler et al., 2002; Zhou et al., 2014). The species sensitivity distribution (SSD) of at-RA was then constructed (Fig. 4) by using a cumulative distribution plotted against rank-assigned centile of $EC_{50}$ of at-RA based on log-normal distribution model (Wheeler et al., 2002; Jin et al., 2012, 2013; Zhou et al., 2014). A hazardous concentration for 5% of species ($HC_5$) was determined to be 0.393 μg at-RA EQ/L (95% CI: 0.046–3.386 μg at-RA EQ/L). The predicted no-effect concentration (PNEC) was determined to be 3.93 ng at-RA EQ/L based on $HC_5$, by applying the acute-to-chronic ratio of 10 and an assessment factor of 10 (Chapman et al., 1998). The fish embryo, *Danio rerio*, was the species most sensitive to exposure to RAs with an $EC_{50}$ value of 1.00 μg at-RA EQ/L, while embryos of the frog, *Rana clamitans,* were the least sensitive with a geometric mean $EC_{50}$ value of 20.9 μg at-RA EQ/L.

Measured environmental concentrations (MEC), used for calculation of hazard quotients (HQs), were expressed as at-RA EQs by using the selected RAEFs for 9c-RA, 13c-RA, at-4-oxo-RA, 9c-4-oxo-RA and 13c-4-oxo-RA (Table 4). To estimate the ecological risks posed by RAs, HQs were calculated by dividing MECs by the PNEC of 3.93 ng at-RA EQ/L. HQs for RAs and 4-oxo-RAs ranged from 0.01 to 1.81 in some rivers in China (Wu et al., 2010; Zhen et al., 2009), while HQs ranged from zero to 2.93 in effluent discharged from STPs in both China and Japan (Zhen et al., 2009; Wu et al., 2010; Sawada et al., 2012; Inoue et al., 2013) (Table 4). HQs for RAs and 4-oxo-RAs in extracellular levels during blooms of cyanobacteria can be as great as 16.41, which was greater than those in rivers and effluents, suggesting that risks were greater during cyanobacterial blooms. It should be noted that all available data




**Fig. 4.** The species sensitivity distribution (SSD) of at-RA was constructed based on the $EC_{50}$ values obtained from acute *in vivo* toxicity tests of five species from literature (See Table 3 for the detailed toxicity values).

**Table 4**
Hazard quotients (HQs) for retinoid acids and their metabolites (expressed as at-RA EQs) detected in freshwater environments and effluents of sewage in China, Japan and Czech Republic.

| Country | Location | MEC (at-RA EQs; ng/L) | HQs | References |
|---|---|---|---|---|
| **Rivers** | | | | |
| China | Rivers adjacent to Liaodong Bay | 0.05–5.42 | 0.01–1.38 | Wu et al. (2010) |
| | Tonghui river and Qing river | 0.5–7.1 | 0.13–1.81 | Zhen et al. (2009) |
| **Sewage Treatment Facilities** | | | | |
| China | Panjin – Effluent | 0–0.06 | 0–0.02 | Wu et al. (2010) |
| | Panjin – Lagoon from duck farm | 0–7.90 | 0–2.01 | Wu et al. (2010) |
| | Beijing – Effluent | 0.5–3.7 | 0.13–0.94 | Zhen et al. (2009) |
| Japan | Osaka – Effluent | 0–0.28 | 0–0.07 | Sawada et al. (2012) |
| | Osaka – Effluent | 0–11.50 | 0–2.93 | Inoue et al. (2013) |
| **Reservoirs, lakes and ponds** | | | | |
| Czech Republic | South Moravia – Extracellular | 0–19.15 | 0–4.87 | Javůrek et al. (2015) |
| | South Bohemia and south Moravia – Extracellular | 0–64.51 | 0–16.41 | Sehnal et al. (2019) |
| China | Taihu Lake – Extracellular | 0–30.11 | 0–7.66 | Wu et al. (2012) |

MEC, measured environmental concentration.
at-RA EQs, sum of the product of the concentration of each retinoid detected in the study multiplied by its respective all-*trans* retinoic acid equivalent factors (RAEFs), which were developed to normalize the concentration of each retinoid to an equivalent concentration of at-RA (Wu et al., 2010; Zhen et al., 2009). It is defined from the $EC_{50}$ of a retinoid.

were obtained from freshwater environments only, although the toxicity data are very limited to a few model species of fish and amphibian and in the absence of information from invertebrate. Toxicity data of RAs and 4-oxo-RAs from marine environments are, however, insufficient to conduct ecological risk assessments of RAs. More studies on the toxic effect of RAs and 4-oxo-RAs on marine species and the detection of their concentrations in seawaters are needed to evaluate the current environmental risks associated with RAs and 4-oxo-RAs in the marine environment.

## 7. Conclusions

This mini-review identified potential sources of retinoic acids (RAs) and their metabolites to aquatic environments, such as excretion from animals, pharmaceutical applications and cyanobacteria. Although these compounds are essential in many biological processes, results of various studies have shown that their excessive intake can result in teratogenic effects in amphibians, fish, gastropods and mammals. Also, RAs and 4-oxo-RAs are unambiguously present in aquatic environments because they are frequently detected in various surface waters around the world. Yet, information on adverse effects of RAs and 4-oxo-RAs to marine organisms and their concentrations in marine environments are largely unknown. Despite the successful development of analytical methods to identify and quantify RAs and 4-oxo-RAs using liquid chromatography–tandem mass spectrometry, there are slightly different extraction procedures from samples that might complicate direct comparability among studies. The predicted no-effect concentration (PNEC) for chronic and continuous exposure to all-*trans* RAs was determined to be 3.93 ng at-RA EQ/L, which was derived from effective concentrations in freshwater species only. With the derived PNEC value from species sensitivity distribution and the measured concentrations of RAs and 4-oxo-RAs in freshwater environments, these chemicals were determined to pose ecological impacts (e.g., malformations, mortality, reproductive impairment), especially to those associated freshwater species such as amphibians, fishes and gastropods. Due to the absence of toxicity data available for marine species, more toxicity tests should be conducted on marine species with wider taxonomic groups, especially for responses to metabolites of RAs, i.e., 4-oxo-RAs. Given that there is a lack of measured environmental concentrations for marine environments, more studies on environmental monitoring of RAs and 4-oxo-RAs should be conducted especially during the occurrence of algal and cyanobacterial blooms and at the discharging point of effluents from STPs. With such essential data, it will be possible to carry out comprehensive ecological risk assessments of RAs and 4-oxo-RAs towards marine environments especially those in highly urbanized coastal cities.

## Declaration of Competing Interest

We declare that we have no conflict of interest.

## Acknowledgements

This work was supported by the Research Grants Council of the Hong Kong SAR Government via the General Research Fund (Project no.: 17126517), the Collaborative Research Fund (project no. C7044-14G) and the Theme-based Research Scheme (project no. T21-711/16-R) to KMYL. GJ Zhou was supported by the Hong Kong Scholars Program (project no.: XJ2012050). Professor Giesy was supported by the "High Level Foreign Experts" program (#GDT20143200016) funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences. He was also supported by the Canada Research Chair program and a Distinguished Visiting Professorship in the School of Biological Sciences of the University of Hong Kong. Klára Hilscherová was supported by the Czech Science Foundation Grant No.18-15199S. The authors thank Kevin Ho for proofreading the draft manuscript, Andy Cheung for drawing the chemical structures, and the anonymous reviewers and editors for their constructive and useful comments.

## References

Allinson, M., Shiraishi, F., Salzman, S.A., Allinson, G., 2011. In vitro assessment of retinoic acid and aryl hydrocarbon receptor activity of treated effluent from 39 wastewater–treatment plants in Victoria, Australia. Arch. Environ. Contam. Toxicol. 61, 539–546.
Alsop, D.H., Brown, S.B., van der Kraak, G.J., 2004. Dietary retinoic acid induces hindlimb and eye deformities in *Xenopus laevis*. Environ. Sci. Technol. 38, 6290–6299.
Amann, P.M., Susic, M., Glüder, F., Berger, H., Krapf, W., Löffler, H., 2015. Alitretinoin (9–*cis* retinoic acid) is effective against pityriasis rubra pilaris: A retrospective clinical study. Acta Derm. Venereol. 95, 329–331.
Balmer, J.E., Blomhoff, R., 2002. Gene expression regulation by retinoic acid. J. Lipid Res. 43, 1773–1808.
Barua, A.B., Furr, H.C., 1998. Properties of Retinoids. In Retinoid Protocols. Humana Press, pp. 3–28.
Chapman, P.M., Fairbrother, A., Brown, D., 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ. Toxicol. Chem. 17 (1), 99–108.
Cicurel, L., Schmid, B.P., 1988. Postimplantation embryo culture for the assessment of the teratogenic potential and potency of compounds. Experientia 44, 833–840.
Collins, M.D., Mao, G.E., 1999. Teratology of retinoids. Annu. Rev. Pharmacol. Toxicol. 39, 399–430.
Degitz, S.J., Kosian, P.A., Makynen, E.A., Jensen, K.M., Ankley, G.T., 2000. Stage– and

species–specific developmental toxicity of all–*trans* retinoic acid in four native North American ranids and *Xenopus laevis*. Toxicol. Sci. 57, 264–274.
Farrell, L.N., Strauss, J.S., Stranieri, A.M., 1980. The treatment of severe cystic acne with 13–*cis*–retinoic acid: evaluation of sebum production and the clinical response in a multiple–dose trial. J. Am. Acad. Dermatol. 3, 602–611.
Franco, P.G., Paganelli, A.R., López, S.L., Carrasco, A.E., 1999. Functional association of retinoic acid and hedgehog signaling in *Xenopus* primary neurogenesis. Development 126 (19), 4257–4265.
Gardiner, D., Ndayibagira, A., Grün, F., Blumberg, B., 2003. Deformed frogs and environmental retinoids. Pure Appl. Chem. 75, 2263–2273.
Gesto, M., Castro, L.F.C., Reis-Henriques, M.A., Santos, M.M., 2012a. Tissue–specific distribution patterns of retinoids and didehydroretinoids in rainbow trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 161, 69–78.
Gesto, M., Castro, L.F.C., Reis-Henriques, M.A., Santos, M.M., 2012b. Retinol metabolism in the mollusk *Osilinus lineatus* indicates an ancient origin for retinyl ester storage capacity. PLoS ONE 7, e35138.
Giraud-Billoud, M., Castro-Vazquez, A., 2019. Aging and retinoid X receptor agonists on masculinization of female *Pomacea canaliculata*, with a critical appraisal of imposex evaluation in the Ampullariidae. Ecotoxicol. Environ. Saf. 169, 573–582.
Gundersen, T.E., Bastani, N.E., Blomhoff, R., 2007. Quantitative high–throughput determination of endogenous retinoids in human plasma using triple–stage liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 21, 1176–1186.
Haga, Y., Suzuki, T., Takeuchi, T., 2002. Retinoic acid isomers produce malformations in postembryonic development of the Japanese flounder, *Paralichthys olivaceus*. Zoolog. Sci. 19, 1105–1112.
Herrmann, K., 1995. Teratogenic effects of retinoic acid and related substances on the early development of the zebrafish (*Brachydanio rerio*) as assessed by a novel scoring system. Toxicol. In Vitro 9, 267–283.
Hoffman, L., Miles, J., Avaron, F., Laforest, L., Akimenko, M.A., 2002. Exogenous retinoic acid induces a stage–specific, transient and progressive extension of Sonic hedgehog expression across the pectoral fin bud of zebrafish. Int. J. Dev. Biol. 46 (7), 949–956.
Horiguchi, T., Ohta, Y., Nishikawa, T., Shiraishi, F., Shiraishi, H., Morita, M., 2008. Exposure to 9–*cis* retinoic acid induces penis and vas deferens development in the female rock shell, *Thais clavigera*. Cell Biol. Toxicol. 24, 553–562.
Hu, L., Zhu, J., Rotchell, J.M., Wu, L., Gao, J., Shi, H., 2015. Use of the enhanced frog embryo teratogenesis assay–*Xenopus* (FETAX) to determine chemically-induced phenotypic effects. Sci. Total Environ. 508, 258–265.
Huang, M.E., Ye, Y.C., Chen, S.R., Chai, J.R., Lu, J.X., Zhao, L., Gu, L.J., Wang, Z.Y., 1988. Use of all–*trans* retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72, 567–572.
Inoue, D., Nakama, K., Sawada, K., Watanabe, T., Matsui, H., Sei, K., Nakanishi, T., Ike, M., 2011. Screening of agonistic activities against four nuclear receptors in wastewater treatment plants in Japan using a yeast two–hybrid assay. J. Environ. Sci. 23, 125–132.
Inoue, D., Nakama, K., Sawada, K., Watanabe, T., Takagi, M., Sei, K., Yang, M., Hirotsuji, J., Hu, J., Nishikawa, J., Nakanishi, T., Ike, M., 2010. Contamination with retinoic acid receptor agonists in two rivers in the Kinki region of Japan. Water Res. 44, 2409–2418.
Inoue, D., Sawada, K., Wada, Y., Sei, K., Ike, M., 2013. Removal characteristics of retinoic acids and 4–oxo–retinoic acids in wastewater by activated sludge treatment. Water Sci. Technol. 67, 2868–2874.
IUPAC–IUB, J.C.O.B.N., 1983. Nomenclature of retinoids. Arch. Biochem. Biophys., vol. 224, pp. 728–731.
Javůrek, J., Sychrová, E., Smutná, M., Bittner, M., Kohoutek, J., Adamovský, O., Nováková, K., Smetanová, S., Hilscherová, K., 2015. Retinoid compounds associated with water blooms dominated by *Microcystis* species. Harmful Algae 47, 116–125.
Jin, X., Wang, Y., Jin, W., Rao, K., Giesy, J.P., Hollert, H., Richardson, K.L., Wang, Z., 2013. Ecological risk of nonylphenol in China surface waters based on reproductive fitness. Environ. Sci. Technol. 48, 1256–1262.
Jin, X., Zha, J., Xu, Y., Giesy, J.P., Richardson, K.L., Wang, Z., 2012. Derivation of predicted no effect concentrations (PNEC) for 2, 4, 6–trichlorophenol based on Chinese resident species. Chemosphere 86, 17–23.
Jonas, A., Buranova, V., Scholz, S., Fetter, E., Novakova, K., Kohoutek, J., Hilscherova, K., 2014. Retinoid–like activity and teratogenic effects of cyanobacterial exudates. Aquat. Toxicol. 155, 283–290.
Jonas, A., Scholz, S., Fetter, E., Sychrova, E., Novakova, K., Ortmann, J., Benisek, M., Adamovsky, O., Giesy, J.P., Hilscherova, K., 2015. Endocrine, teratogenic and neurotoxic effects of cyanobacteria detected by cellular in vitro and zebrafish embryos assays. Chemosphere 120, 321–327.
Kaya, K., Shiraishi, F., Uchida, H., Sano, T., 2011. A novel retinoic acid analogue, 7–hydroxy retinoic acid, isolated from cyanobacteria. BBA 1810, 414–419.
Lambert, W.E., De Leenheer, A.P., 1985. Demonstration of retinoic acid isomers in human urine under physiological conditions. Experientia 41, 359–360.
Lucek, R.W., Colburn, W.A., 1985. Clinical pharmacokinetics of the retinoids. Clin. Pharmacokinet. 10, 38–62.
Maiani, G., Periago Castón, M.J., Catasta, G., Toti, E., Cambrodón, I.G., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D., Schlemmer, U., 2009. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Mol. Nutr. Food Res. 53 (S2), S194–S218.
Miles, J.A., Machattou, P., Nevin-Jones, D., Webb, M.E., Millard, A., Scanlan, D.J., Taylor, P.C., 2019. Identification of a cyanobacterial aldehyde dehydrogenase that produces retinoic acid in vitro. Biochem. Biophys. Res. Commun. 510, 27–34.
Nishikawa, J.I., Mamiya, S., Kanayama, T., Nishikawa, T., Shiraishi, F., Horiguchi, T., 2004. Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. Environ. Sci. Technol. 38, 6271–6276.
Novák, J., Benísek, M., Hilscherová, K., 2008. Disruption of retinoid transport, metabolism and signaling by environmental pollutants. Environ. Int. 34, 898–913.
Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65, 995–1010.
Peck, G.L., Olsen, T.G., Butkus, D., Pandya, M., Arnaud-Battandier, J., Gross, E.G., Windhorst, D.B., Cheripko, J., 1982. Isotretinoin versus placebo in the treatment of cystic acne: a randomized double–blind study. J. Am. Acad. Dermatol. 6, 735–745.
Pipal, M., Priebojova, J., Koci, T., Blahova, L., Smutna, M., Hilscherova, K., 2020. Field cyanobacterial blooms producing retinoid compounds cause teratogenicity in zebrafish embryos. Chemosphere 241, 125061.
Priebojová, J., Hilscherová, K., Procházková, T., Sychrová, E., Smutná, M., 2018. Intracellular and extracellular retinoid–like activity of widespread cyanobacterial species. Ecotoxicol. Environ. Saf. 150, 312–319.
Ritchie, H.E., Brown—Woodman, P.D., Korabelnikoff, A., 2003. Effect of co–administration of retinoids on rat embryo development in vitro. Birth Defects Res. Part A: Clin. Mol. Teratol., vol. 67, pp. 444–451.
Ross, S.A., McCaffery, P.J., Drager, U.C., De Luca, L.M., 2000. Retinoids in embryonal development. Physiol. Rev. 80, 1021–1054.
Sawada, K., Inoue, D., Wada, Y., Sei, K., Nakanishi, T., Ike, M., 2012. Detection of retinoic acid receptor agonistic activity and identification of causative compounds in municipal wastewater treatment plants in Japan. Environ. Toxicol. Chem. 31, 307–315.
Sehnal, L., Procházková, T., Smutná, M., Kohoutek, J., Lepšová-Skácelová, O., Hilscherová, K., 2019. Widespread occurrence of retinoids in water bodies associated with cyanobacterial blooms dominated by diverse species. Water Res. 156, 136–147.
Selderslaghs, I.W., Blust, R., Witters, H.E., 2012. Feasibility study of the zebrafish assay as an alternative method to screen for developmental toxicity and embryotoxicity using a training set of 27 compounds. Reprod. Toxicol. 33, 142–154.
Selderslaghs, I.W., Van Rompay, A.R., De Coen, W., Witters, H.E., 2009. Development of a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo. Reprod. Toxicol. 28, 308–320.
Smutná, M., Priebojová, J., Večerková, J., Hilscherová, K., 2017. Retinoid–like compounds produced by phytoplankton affect embryonic development of *Xenopus laevis*. Ecotoxicol. Environ. Saf. 138, 32–38.
Stark, D.R., Gates, P.B., Brockes, J.P., Ferretti, P., 1998. *Hedgehog* family member is expressed throughout regenerating and developing limbs. Develop. Dynam.: An Off. Publ. Am. Assoc. Anatom. 212 (3), 352–363.
Summerbell, D., 1983. The effect of local application of retinoic acid to the anterior margin of the developing chick limb. Development 78, 269–289.
Sychrová, E., Priebojová, J., Smutná, M., Nováková, K., Kohoutek, J., Hilscherová, K., 2016. Characterization of total retinoid–like activity of compounds produced by three common phytoplankton species. Harmful Algae 60, 157–166.
van Breemen, R.B., Nikolic, D., Xu, X., Xiong, Y., van Lieshout, M., West, C.E., Schilling, A.B., 1998. Development of a method for quantitation of retinol and retinyl palmitate in human serum using high–performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry. J. Chromatogr. A 794, 245–251.
Villeneuve, D.L., Blankenship, A.L., Giesy, J.P., 2000. Derivation and application of relative potency estimates based on in vitro bioassay results. Environ. Toxicol. Chem. 19, 2835–2843.
Völker, J., Castronovo, S., Wick, A., Ternes, T.A., Joss, A., Oehlmann, J., Wagner, M., 2016. Advancing biological wastewater treatment: extended anaerobic conditions enhance the removal of endocrine and dioxin–like activities. Environ. Sci. Technol. 50, 10606–10615.
Wang, Y., Chang, W.Y., Prins, G.S., van Breemen, R.B., 2001. Simultaneous determination of all–*trans*, 9–*cis*, 13–*cis* retinoic acid and retinol in rat prostate using liquid chromatography–mass spectrometry. J. Mass Spectrom. 36, 882–888.
Wang, Y., Chen, J., Du, C., Li, C., Huang, C., Dong, Q., 2014. Characterization of retinoic acid–induced neurobehavioral effects in developing zebrafish. Environ. Toxicol. Chem. 33, 431–437.
Wang, Z.Y., Chen, Z., 2008. Acute promyelocytic leukemia: From highly fatal to highly curable. Blood 111 (5), 2505–2515.
Wheeler, J.R., Grist, E.P.M., Leung, K.M.Y., Morritt, D., Crane, M., 2002. Species sensitivity distributions: Data and model choice. Mar. Pollut. Bull. 45, 192–202.
Wu, X., Hu, J., Jia, A., Peng, H., Wu, S., Dong, Z., 2010. Determination and occurrence of retinoic acids and their 4–oxo metabolites in Liaodong Bay, China, and its adjacent rivers. Environ. Toxicol. Chem. 29, 2491–2497.
Wu, X., Jiang, J., Hu, J., 2013. Determination and occurrence of retinoids in a eutrophic lake (Taihu Lake, China): Cyanobacteria blooms produce teratogenic retinal. Environ. Sci. Technol. 47, 807–814.
Wu, X., Jiang, J., Wan, Y., Giesy, J.P., Hu, J., 2012. Cyanobacteria blooms produce teratogenic retinoic acids. Proc. Natl. Acad. Sci. 109, 9477–9482.
Yu, L., Zhang, X., Yuan, J., Cao, Q., Liu, J., Zhu, P., Shi, H., 2011. Teratogenic effects of triphenyltin on embryos of amphibian (*Xenopus tropicalis*): a phenotypic comparison with the retinoid X and retinoic acid receptor ligands. J. Hazard. Mater. 192, 1860–1868.
Zhen, H., Wu, X., Hu, J., Xiao, Y., Yang, M., Hirotsuji, J., Nishikawa, J.-I., Nakanishi, T., Ike, M., 2009. Identification of retinoic acid receptor agonists in sewage treatment plants. Environ. Sci. Technol. 43, 6611–6616.
Zhou, G.J., Li, X.Y., Leung, K.M.Y., 2019. Retinoids and oestrogenic endocrine disrupting chemicals in saline sewage treatment plants: removal efficiencies and ecological risks to marine organisms. Environ. Int. 127, 103–113.
Zhou, G.J., Wang, Z., Lau, E.T.C., Xu, X.R., Leung, K.M.Y., 2014. Can we predict temperature–dependent chemical toxicity to marine organisms and set appropriate water quality guidelines for protecting marine ecosystems under different thermal scenarios? Mar. Pollut. Bull. 87, 11–21.
Zhu, J., Yu, L., Wu, L., Hu, L., Shi, H., 2014. Unexpected phenotypes of malformations induced in *Xenopus tropicalis* embryos by combined exposure to triphenyltin and 9–*cis*–retinoic acid. J. Environ. Sci. 26, 643–649.