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Review

Occurrence, toxic effects and removal of metformin in the aquatic environments in the world: Recent trends and perspectives

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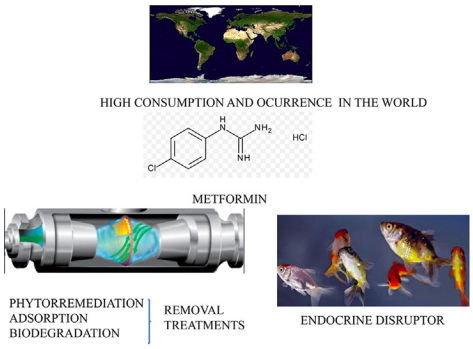
HIGHLIGHTS

- MET antidiabetic drug review was conducted.
- The annual consumption of MET worldwide is in tons.
- The occurrence of MET in the world ranges from ng L^{-1} to μg L^{-1}.
- MET toxic effects are embryotoxicity, teratogenicity and endocrine disruption.
- Phytoremediation, adsorption and biodegradation are used to remove MET.

GRAPHICAL ABSTRACT



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ABSTRACT

Metformin (MET) is the most common drug used to treat type 2 diabetes, but also it is used as an anti-cancer agent and as a treatment for polycystic ovary syndrome. This drug is not metabolized in the human body, and may enter into the environment through different pathways. In wastewater treatments plants (WWTPs), this contaminant is mainly transformed to guanylurea (GUA). However, three further transformation products (TPs): (a) 2,4- diamino-1,3,5-triazine, 4-DAT; (b) 2-amino-4-methylamino-1,3,5-triazine, 2,4-AMT; and (c) methylbiguanide, MBG; have also been associated with its metabolism. MET, GUA and MBG have been found in WWTPs influents, effluents and surface waters. Furthermore, MET and GUA bioaccumulate in edible plants species, fish and mussels potentially contaminating the human food web. MET is also a potential endocrine disruptor in fish. Phytoremediation, adsorption and biodegradation have shown a high removal efficiency of MET, in laboratory. Nonetheless, these removal methods had less efficiency when tried in WWTPs. Therefore, MET and its TPs are a threat to the human being as well as to our environment. This review comprehensively discuss the (1) pathways of MET to the environment and its life-cycle, (2) occurrence of MET and its transformation products (3) removal, (4) toxic effects and (5) future trends and perspectives of possible methods of elimination in water in order to provide potential options for managing these contaminants.

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Contents

1. Introduction

Diabetes mellitus is a metabolic disorder that occurs when the body cannot produce enough insulin or cannot use this hormone effectively, leading to consistent hyperglycemia (Chellappan et al., 2018). Currently, over 451 million people worldwide are estimated to have diabetes, and approximately 87% to 91% of all people with diabetes suffer type 2 diabetes (T2D) (Cho et al., 2018).

MET is the first-line oral therapy and the most commonly prescribed oral agent for T2D (Foretz et al., 2014; Rena et al., 2013). It is currently thought to be the highest drug by weight released into the aquatic environment. In the US, prescriptions of MET increased, more than 56% in an eight-year period, from 49.2 million in 2007 to 76.9 million in 2014 (Kleinrock, 2012; Aitken et al., 2015). Similarly, the prescriptions in Netherlands and Western Europe prescriptions also increased by 26% between 2008 and 2012 (ter Laak and Baken, 2014). Furthermore, it is expected that MET consumption steadily increase in the upcoming years, as numerous studies suggest MET usage as an anticancer agent (Mallik and Chowdhury, 2018; Morales and Morris, 2015). Likewise, MET has been also associated with regularization of the menstrual cycle and most hormonal profiles in women with polycystic ovary syndrome (PCOS) (Yang et al., 2018) which also, may further intensify the global use of MET.

Due to its high consumption, low octanol-water partition coefficient (K_{ow}), and its null metabolism by the human body (Gong et al., 2012). MET is expected to be present worldwide in wastewater treatment plants (WWTPs) effluents, and surface waters. However, during its pass through the WWTPs, MET is biologically transformed to guanylurea (GUA). A bacterial double dealkylation (where both methyl groups are removed at the terminal nitrogen) has been proposed as the main mechanism for the conversion of MET to GUA in WWTPs (Markiewics et al., 2017a).

Although, some studies have shown a direct correlation between the degradation of MET and the production of GUA. Some others have shown that GUA concentrations in effluents are significantly lower than MET concentrations in influents (Kosma et al., 2015), but also that GUA concentrations are higher in influents than in effluents (Tisler and Zwiener, 2018). This may be explained by the formation and subsequent degradation of intermediates metabolites. For example, 3 further transformation products (TPs) of MET: a) 2,4- diamino-1,3,5-triazine, 2,4-DAT; b) 2-amin o-4-methylamino-1,3,5-triazine, 2,4-AMT; c) methylbiguanide, MBG) have been detected in WWTPs effluents and in surface waters (Tisler and Zwiener, 2018).

In view of the fact that MET and its TPs are often found at high concentrations in the aquatic environment and can exhibit different toxicities in non-target organisms, a timely review seems appropriate. The aim of this work was to comprehensively investigate the occurrence of MET and its four TPs in influents and effluents of WWTPs, surface water, groundwater and sludge. Additionally, we evaluate and compare treatments involved in the removal of MET and finally, we discuss the toxic effects of

Table 1
Prescriptions of metformin in 2017.

Country	Prescriptions per year	Source
US	81,305,416	MEPS, 2018
England	21,163,271	Prescribing and Medicines Team Health and Social Care Information Centre, 2018.
North Ireland	435,432	Mulholland, 2018.
Wales	1,397,814	National Statistics Ystadegau Gwladol, 2018.
Scotland	1,249,597	Information Services Division National Services Scotland, 2018
Denmark	1,589,000	Sundhedsdata-Styrelsen, 2018.
Netherlands	6,146,557	Zorginstituut Nederland, 2018.
Sweden	1,499,590	Socialstyrelsen, 2018.

° prescriptions in 2016

Table 2
Worldwide occurrence of metformin and its transformation products.

Emerging pollutant	Country	Concentrations (μg/L)							Source
		WWTP Influent (min.–max.)	WWTP Effluent (min. –max.)	Surface Water (min. –max.)	Drinking Water (min. –max.)	Sludge (μg/g) (min. –max.)	Groundwater (min. –max.)	Hospital Effluent (min. –max.)	
Metformin	Greenland	n.a	3.58–6.8	0.0331–0.748	n.a	0.455–0.553	n.a	n.a	Huber et al., 2016
	Canada	n.a	0.067–10.608	0.012–1.487	n.a	n.a	n.a	n.a	Ghoshdastidar et al., 2015
		n.a	n.a	0.145–10.1	n.a	n.a	n.a	n.a	De Solla et al., 2016
	USA	n.a–99	n.a	n.a	n.a	n.a	n.a	n.a	Blair et al., 2015
		6.06–720	0.401–58.9	n.a	n.a	n.a	n.a	0.009–630	Oliveira et al., 2015
		n.a	29.3–82.7	0.105–0.832	n.a	n.a	n.a	n.a	Meador et al., 2016
		n.a	n.a	0.0014–2.635	n.a	n.a	n.a	n.a	Bradley et al., 2016
		n.a	n.a	0.0104–4.308	n.a	n.a	n.a	n.a	Bradley et al., 2017a
		n.a	n.a	0.00239–0.281	n.a	n.a	n.a	n.a	Bradley et al., 2017b
		n.a	n.a	0.0105–0.903	n.a	n.a	n.a	n.a	Elliott et al., 2017
		n.a	n.a	n.a–33.6	n.a	n.a	n.a	n.a	Elliott et al., 2017
		n.a	n.a	n.a–0.21	n.a	n.a	n.a	n.a	Weissinger et al. 2018
		n.a	n.a	n.a–7.13	n.a	n.a	n.a	n.a	Bai et al., 2018
		36.1–73.3	2.6–9.6	n.a	n.a	n.a	n.a	n.a	Xing et al., 2018
	Mexico	40.7–94.6	3.01–3.77	n.a	n.a	n.a	n.a	n.a	Estrada-Arriaga et al., 2016
		13.4–32.1	0.0576–0.21	n.a	n.a	n.a	n.a	n.a	Estrada-Arriaga et al., 2016
		n.a	n.a	n.a	n.a	n.a	0.0103–107	n.a	Lesser et al., 2018
		n.a	n.a	n.a	n.a	n.a	n.a	1.29–1.33	Pérez-Alvarez et al., 2018
		n.a	n.a	n.a	n.a	n.a	n.a	1.36–1.48	Luja-Mondragón et al., 2019
	Brazil	n.a	n.a	n.a	n.a	n.a	n.a	1.7–2.3	Chiarello et al., 2016
	Iceland	1.79–59	0.234–5.59	n.a	n.a	0.149–7.81	n.a	n.a	Huber et al., 2016
	Faroe Islands	4.15–9.66	7.42–7.56	0.0614–0.0779	n.a	0.239–0.31	n.a	n.a	Huber et al., 2016
	Poland	3.8187–16.7907	0.0075–0.0629	n.a	0.0017–0.008	n.a	n.a	n.a	Kot-Wasik et al., 2016
	Germany	86.2–142.3	3.4–6.4	0.001–0.643	n.a	n.a	n.a	n.a	Trautwein et al., 2014
		n.a	n.a	<0.46–1.66	n.a	n.a	n.a	n.a	Posselt et al., 2018
		14–95	0.7–6.5	<0.001–0.47	n.a	n.a	n.a	n.a	Tisler, & Zwiener, 2018
	Spain	n.a–5.927	n.a–1.252	n.a–0.013	n.a	n.a	n.a	n.a	Carmona et al., 2017
	Portugal	70–325	0.05–58	n.a	n.a	n.a	n.a	n.a	de Jesus Gaffney et al., 2017
	Moldova	n.a	n.a	0.1–0.24	n.a	n.a	n.a	n.a	Moldovan et al., 2018
	Romania	n.a	n.a	n.a–0.44	n.a	n.a	n.a	n.a	Moldovan et al., 2018
	Greece	n.a	n.a	n.a	n.a	0.0414–0.0782	n.a	n.a	Gago-Ferrero et al., 2015
		<0.0251–1.167	<0.0167–0.026	n.a	n.a	n.a	n.a	n.a	Kosma et al., 2015
		n.a	n.a	n.a	n.a	0.147–0.237	n.a	n.a	Thomaidi et al., 2016
	Turkey	n.a	n.a	<0.00014–0.0141	n.a	n.a	n.a	n.a	Guzel et al.,2018
	Saudi Arabia	4.02–31.2	<3–4.51	n.a	n.a	n.a	n.a	n.a	Shraim et al., 2017
		n.a	n.a	0.007–4.8009	n.a	n.a	n.a	n.a	Ali et al.,2017
	China	n.a	n.a	0.051–2.917	n.a	n.a	n.a	n.a	Kong et al., 2015
		n.a	n.a	n.a	n.a	n.a	n.a–0.045	n.a	Kong et al., 2016

(*continued on next page*)

Table 2 (*continued*)

Emerging pollutant	Country	Concentrations (μg/L)							Source
		WWTP Influent (min.–max.)	WWTP Effluent (min. –max.)	Surface Water (min. –max.)	Drinking Water (min. –max.)	Sludge (μg/g) (min. –max.)	Groundwater (min. –max.)	Hospital Effluent (min. –max.)	
		21–35	0.01–0.64	0.0016 – 5.8	n.a	n.a	n.a	n.a	Yao et al., 2018
		n.a	n.a	0.0002–0.1214	n.a	n.a	n.a	n.a	Asghar et al., 2018
		2.42–53.6	n.a	n.a	n.a	n.a	n.a	n.a	Yan et al., 2019
	Vietnam	n.a	n.a	0.01–8.247	n.a	n.a	n.a	n.a	Chau et al., 2018
	Egypt	n.a	0.168–5.61	0.021–0.063	n.a	n.a	n.a	n.a	Nguyen, 2018
	Cameroon	n.a	n.a	n.a	n.a	n.a	n.a	n.a–0.154	Mayoudom et al., 2018
	South Africa	3.585–9.228	0.167–0.566	0.065–0.316	n.a	n.a	n.a	n.a	Archer et al., 2017
Guanylurea	Germany	0.9–2.0	28.2–67.2	0.004–0.391	n.a	n.a	n.a	n.a	Trautwein et al., 2014
		158–2100	26–810	<0.01–4.502	n.a	n.a	n.a	n.a	Tisler, & Zwiener, 2018
		n.a	n.a	15–222	n.a	n.a	n.a	n.a	Posselt et al., 2018
	Greece	<0.0196–0.084	<0.0283–0.627	n.a	n.a	n.a	n.a	n.a	Kosma et al., 2015
	China	0.004–5.4	15–28	0.0012–3.5	n.a	n.a	n.a	n.a	Yao et al., 2018
Methylbiguanide	Germany	n.a	0.035–0.122	<0.01–0.031	n.a	n.a	n.a	n.a	Tisler, & Zwiener, 2018

n.a: not available; bql: below quantification limit.

MET and its TPs towards different aquatic organisms (dahpnids, fish, etc.).

2. Pathways to the environment: Life-cycle of metformin

The lifecycle begins with the manufacture of MET, which can lead to its direct discharge into liquid or solid waste systems. The manufactured MET is transported and distributed to hospital pharmacies, where is prescribed for patients with T2D, cancer and women with PCOS. Table 1 summarized MET consumption data of some countries in terms of prescriptions issued. Despite the increasing prevalence of diabetes worldwide, there is a currently huge knowledge gap about the consumption patterns of this anti-diabetic drug. In the future, it will be helpful to study its consumption pattern in order to provide a better diabetes control as well as to have better understanding and control on its environmental discharges.

Once administered MET is excreted unaltered form (Gong et al., 2012). Thereby, this waste material enter to the sewer system, where MET is partially transformed to GUA due to bacteria adaptation in the sewage pipes. Finally, this waste material is collected in WWTP, and either directly discharged untreated into the environment or passed through one or more treatment steps before being discharged as effluent into the natural aquatic environment.

If MET is treated in WWTPs, it can be bacterially transformed to its main TPs (GUA, MBG, 2,4-AMT, 2,4-DAT) (Tisler and Zwiener, 2018). Chlorination, phytoremediation and adsorption in graphene oxide (GO) are the only techniques that have shown high rates of removal (Quintão et al., 2016; Cui and Schröder, 2016; Moogouei et al., 2018; Zhu et al., 2017). However, these treatments are not applied in all WWTPs. In consequence, huge amounts of MET and GUA are released in to the aquatic environment where they can exhibit different toxicities in non-target organisms.

Other pathways for the entrance of MET into the environment or even worst into the human food web, are the use of sewage sludge as soil fertilizer and conditioner for plant growth, or the irrigation of fields directly with wastewater (Eggen and Lillo, 2012; Lesser et al., 2018).

3. Occurrence of metformin and its transformation products

MET and GUA have a K_{ow} of −4.3 and −2.5 at pH 7.4, respectively (ter Laak and Baken, 2014). Based on these properties, distribution in the aqueous phase for MET and GUA is expected to be high, which is demonstrated by its ubiquitous presence in surface, ground and drinking water.

Table 2 summarize the data collected from literature regarding the occurrence of MET and its TPs about its presence in the aquatic environment. Data collected have dates between 2014 and 2019, and will be discussed in the subsequent sections.

3.1. Wastewater

The maximum concentration of MET in WWTP influents was 702 μg L^{-1}, in US (Oliverira et al., 2015). This value correspond to a WWTP that recives daily 8% effluents from a one mid-size hospital, with approximately 600 beds.

Other countries with high concentrations of MET in influents were Portugal with 325 μg L^{-1} (de Jesus Gaffney et al., 2017) and Germany with 142.3 μg L^{-1} (Trautwein et al., 2014).

In Portugal, the WWTP receive urban and industrial wastewater from a combined sewage network, which may justifies the high influent concentrations of MET. However, what is even more remarkable is that MET values in WWTP effluents still being high.

Table 3
Removal of MET and its transformation products by various processes.

Method	Material	Metformin Concentrations	Time	Other conditions	Results	Source
Adsorption	0.003 g GO	10 mg/L	2.66 h	14.85–44.85 °C pH: 4–11	Qe: 47.1 mg/g Ka: 0.0007 g/mg•min RE: 80% in 0.33 h Uptake ↑ (pH: 4–6) ΔG^o and ΔH^o: - ΔS^o: +	Zhu et al., 2017
	0.05 g TNTs	30 mg/L	3 h	27 °C pH: 6 Groundwater	Qe: 29.69 mg/g Ka: 0.6120 g/mg•min RE ↓ Qe: 4.66 mg/g Ka: 1.1008 g/mg•min	Orona-Návar et al., 2018
Biodegradation	0.4 g/L sludge	25–30 mg/L * 25–30 mg/L	40w 40w	n.a n.a	MET was completely degraded after 15 days GUA detected No significant decrease was observed after 27 days	Markiewicz et al., 2017a
	0.4 g/L sludge	20 mg/L MET	25d	Aerobic20 °C	MET reached an average degradation of 76% The mineralization ranged from 0% to 51%	Markiewicz et al., 2017b
	0.5 mL sludge	215 mg/L (MET) + 245.2 mg/L (GUA) + 5000 mg/L (GLU) 245.2 mg/L (GUA) + 5000 mg/L (GLU) * 245.2 mg/L 215 mg/L (MET) + 5000 mg/L (GLU9 215 mg/L	30d 20d 20d 10d 10d	Aerobic 30 °C pH: 7 Aerobic 30 °C pH: 7 Aerobic 30 °C pH: 7 Aerobic 30 °C pH: 7 Aerobic 30 °C pH: 7	40% of GUA degraded after 4d 34% of MET was degraded from day 20 to 30 (no further degradation) GUA was completely degraded after 12 days 25% of GUA was degraded after 19 days MET was completely degraded after 7 days GUA reached a maximum of 111.3 mg/L after 9 days MET was completely degraded after 9 days GUA bdl	Briones et al., 2018
	3 g/L sludge 8 g/L sludge 30 g/L sludge	5 mg/L (MET) + 13 mg/L (GUA) 40 mg/L (MET + 40 mg/L GUA) 9 mg/L 9 mg/L 6 mg/L * 8 mg/L	n.a n.a n.a n.a n.a n.a	Aerobic 22 °C Aerobic 22 °C Aerobic 22 °C Anaerobic 22 °C Anaerobic 22 °C Anaerobic 22 °C	99% of MET degraded after 12 days GUA reached 90% of MET concentration after 12 days 98% of GUA degraded after 45 days MBG reached a maximum of 0.05 μmol/L after 7 days MBG was no longer detectable after 17 days 2,4-AMT reached a maximum of 0.37 μmol/L after 7 days After 45 days 0.08 μmol/L of 2,4-AMT were still detectable GUA was completely degraded after 47 days MBG show similar trend 2,4-AMT reached the maximum amount after 21 days 2,4-DAT detected MET was completely transformed into GUA after 5 days GUA was completely degraded after 36 days 2,4-AMT reached a maximum of 4 μmol/L after 14 days 57% of 2,4-AMT was incomplete degraded after 24 days MET was completely degraded after 40 days GUA was completely degraded after 17 days MBG and 2,4-AMT were completely degraded after 40 days GUA was completely degraded after 8 days	Tisler and Zwiener, 2019

(*continued on next page*)

Table 3 (*continued*)

Method	Material	Metformin Concentrations	Time	Other conditions	Results	Source
Photodegradation	UV-C	10 mg/L	30 min	- 120 mg/L of TiO2	9.2% of MET was degraded0.72% if mineralization 31% of MET was degraded	Quintão et al., 2016
	UV254	2 μmol/L	70 min	21.85 °C 300–600 μM 21.85 °C 300–600 μM 30–60 μM 21.85 °C	K: 0.0037/min Kd: 0.0029–0.0152/min Kd: 0.0038–0.0055/min	Neamțu et al., 2014
Phytoremediation	Typha latifolia	6.5–32.3 mg/L MET	28d	n.a	R: 74–81.1% Ka: 0.0631–0.0969/d MBG increased at a maximum concentration of 26.7 nmol/g	Cui and Schröder et al., 2016
	Amaranthus retroflexus Phragmites australis Ricinus communis Brassica napus Celosia cristata Helianthus annuus	20 mg/L MET 50 mg/L MET	14d	pH: 5.5	R: 63% R: 58.4% R: 50.03% R: 35.7% R: 45.06% R: 21% R: 31.06% R: 56.98% R: 58% R: 58.1% R: 69.53% R: 65.7%	Moogouei et al., 2018
Other	10 mg/L NaClO 8 mg/L Ozone	10 mg/L	30 min	23 °C -	60% of MET was degraded 60% of MET was degraded 20% of mineralization	Quintão et al., 2016
	60 mg/L SME + 20 mg/L CS	230.8 ng/L (WWTP)	1 h	pH: 7	21% of MET was degraded	Mohd Amin et al. (2016)

* Guanylurea concentrations; n.a: not available; Ks: sorption coefficient; D: desorption; GO: graphene oxide; Qa: adsorption quantity; RE: removal efficiency; TNTs: titanate nanotubes; Ka: adsorption rate; Kd: degradation rate constant; MWHC: Maximum water holding capacity; bdl: below detected; R: remediation; SME: Smectite; CS: clay starch.

This could be because effluents were treated with a biological system, which is known to be poorly efficient in the removal of MET.

On the other hand, in Germany, Trautwein et al. (2014), reported influent and efluent concentrations of a WWTP designed for 600,000 population equivalent. Although this WWTP also use a biological treatment, MET was removed in a 95.5%, reaching a maximum concentration of 6.4 μg L^{-1}. Nevertheless, GUA was detected in concentrations of concern, with 67.2 μg L^{-1} in effluents.

More recently Tisler and Zwiener (2018) reported GUA concentrations were higher than MET concentrations. In this case, there was no direct correlation between MET degradation and GUA formation, since only 25% on molar basis of degraded MET could be found as GUA. This indicates there could be other biotic or abiotic removal processes for MET which may form other TPs such as: MBG, 2,4-DAT and 2,4-AMT. In fact, in this study MBG was detected in the effluents with a concentration of 0.122 μg L^{-1}, and even though 2,4-DAT and 2,4-AMT were not quantified, both compounds showed similar increasing response trends, with higher concentrations in the effluent than in the influent.

3.2. Surface water

Increasing evidence suggests MET and its TPs pass through or are formed in WWTPs, which means effluents are the major source of these contaminants in surface water. Once MET and its TPs enter the environment, its presence, persistence and quantity are largely determined by the volume of consumption, removal rate in WWTPs and by a dilution factor in surface water. Hence, it is expected, the concentration of MET and its TPs will be lower in receiving water bodies.

The highest concentration of MET in surface water was found in US by Elliott et al. (2017). They collected a total of 292 surface-water samples from 12 US tributaries. From all these samples, MET reached a detection frequency of 71% and a maximum concentration of 33.6 μg L^{-1}.

Nonetheless, as it was expected, higher concentrations of GUA have been found in surface water. For instance, in Germany, Posselt et al. (2018) collected multiples samples from Erpe River, which receives effluent water from several smaller WWTPs and one large WWTP. Between all the drugs quantified, GUA reached a maximum concentration of 222 μg L^{-1}.

3.3. Groundwater

Groundwater contamination has become a growing public concern, because of the dramatically increasing fresh water demand. However, compared to the numerous efforts undertaken to evaluate contamination of surface water, the quality of groundwater is relatively poorly understood. Thus, there is little information available on groundwater quality, particularly with respect to MET and its TPs.

From 2014 to 2019, only two studies reported concentrations of MET on groundwater. Kong et al. (2016) sampled 17 domestic wells in North China, and reported a maximum concentration of 0.045 μg L^{-1}, with a 7.4% of detection frequency, and Lesser et al. (2018) collected samples from 17 groundwater wells of the Mezquital Valley, which receives more than 70% of the wastewater generated by Mexico City Metropolitan Area (21 million inhabitants). Here, MET reached a maximum concentration of 0.029 μg L^{-1}.

3.4. Drinking water

Groundwater is the most reliable source of public drinking water in many regions of the world. Nonetheless, surface and ground water are closely related, and can transfer contaminants

from one to another. Thereby, groundwater resources are increasingly threatened by chemical contaminants like MET and its TPs, and could potentially be transferred to humans through drinking water.

In Poland, Kot-Wasik et al. (2016) studied treated water, which is basically drinking water, from a water treatment plant (WTP). This water supplies many districts and several villages in Poland. According to their results, MET reached a 64% of detection frequency and a maximum concentration of 0.008 μg L^{-1}.

3.5. Sludge

In extend to our knowledge to date only, three studies have reported the presence of MET on sludge. Huber et al. (2016) collected sludge samples from three different countries, Iceland, Greenland and Faroe Island, and reported 7.81 μg g^{-1}, 0.55 μg g^{-1} and 0.31 μg g^{-1} concentrations of MET, respectively. The other two studies were performed in Greece. Gago-Ferrero et al. (2015) collected sewage sludge from five WWTPs and an island, in this study MET reached a maximum concentration of 0.078 μg g^{-1}.

Lastly, Thomaidi et al. (2016) investigated the occurrence of 50 pharmaceuticals, included MET, in samples were collected at the Athens STP. MET was quantified with a maximum concentration of 0.23 μg g^{-1}.

As MET is widely use for the treatment of diabetes, and its consumption has risen steadily in the last years. Hospital activities and pharmaceutical manufacturers are the major source of these contaminants via effluent. It is recommended that healthcare and pharmaceutical facilities monitor and assess their discharges in order to reduce the loading of MET to sensitive water bodies. MET and GUA have been found in high concentrations in effluents and surface waters. This because, most of the countries lack of efficient techniques to remove these contaminants from WWTPs. Future works should try to enhance the efficiency of these old techniques or develop new techniques with high removal rates.

Finally, little information is known about the occurrence of GUA and other TPs in the aquatic environment. Future works should stimulate research to understand the potential risk of these contaminants to the water bodies.

4. Removal

Overall, the mechanisms involved in the removal of pharmaceuticals in WWTPs are adsorption, biodegradation, abiotic degradation and phytoremediation. Due to its low K_{ow}, MET and GUA are expected to be removed in WWTPs mainly through phytoremediation.

The following section briefly discusses the removal efficiency of MET achieved under different treatments techniques. Additionally, Table 3 summarizes the removal rates of MET during each treatment process.

4.1. Adsorption of MET

Graphene oxide (GO) can be used as an adsorbent to remove MET from water, Zhu et al. (2017) carried out a study under different temperatures and pH values. In this study, GO exhibited a high adsorption capacity, with an 80% of MET removed within 20 min. However, the adsorption capacity was strongly dependent on temperature, pH and ionic strength.

Orona-Návar et al. (2018) evaluated the adsorption behavior of six organic pollutants, using titanate nanotubes. MET was quickly adsorbed onto nanotubes, when the compounds was dissolved in ultra-pure water. However, removal efficiency decreased in ground water matrices, due to the presence of other ions, which interact with the active sites at the titanate nanotubes.

4.2. Biodegradation of MET

Markiewicz et al. (2017a) used activated sewage sludge to tested primary biodegradation of MET, GUA, and other antidiabetic drugs. MET was completely eliminated in 15 days, with the gradual appearance of GUA. However, one of the replicates exhibited a long lag phase, indicating a two-step conversion of MET to GUA. A double dealkylation was proposed as a possible degradation pathway of MET degradation.

Briones et al. (2018) used glucose (GLU) as co-substrate to enrich cultures with specific degraders of MET and GUA. As a single substrate, GUA only reached a 25% of degradation after 19 days. However, in the presence of GLU, it was completely degraded, after

Table 4
Bioconcentration and bioaccumulation of metformin and guanylurea.

Species	Metformin Concentrations (μg/g)(min–max)	BCF or BAF	Source
Hordeum vulgare	n.a–5.0 * n.a–2.65	BCF: 0.91	Eggen and Lillo, 2012
Vicia faba	n.a–4.85 * n.a–4.25	BCF: 0.88	
Solanum tuberosum	13.27–15.83 * 2.60–5.66	BCF: 2.41	
Avena sativa	n.a	BCF: 1.35	
Brassica rapa	n.a	BCF: 21.72	
Brassica napus	n.a	BCF: 20.63	
Daucus carota	n.a	BCF: 1.50–3.52	
Solanum lycopersicum	n.a	BCF: 0.02–0.06	
Cucurbita pepo	n.a	BCF: 0.12–0.18	
Triticum aestivum	n.a	BCF: 0.29	
Lasmigona costata	n.a–0.00665	BAF: 0.66	De Solla et al., 2016
Typha latifolia	1.2913–1,462.13	BAF: 0.09–53.34	Cui and Schröder, 2016
Leptocottus armatus	n.a–0.028	n.a	Meador et al., 2017
	n.a–0.0278	BCF:1.42	Meador et al., 2018
Oncorhynchus tshawytscha	n.a–0.04	n.a	Yeh et al., 2017
	n.a–0.0395	BCF:1.42	Meador et al., 2018
Oryzias latipes	n.a–3,120	n.a	Ussery et al., 2018
Leptophlebidae	0.0402–0.312	n.a	Althakafy et al., 2018
Economidae	n.a–0.0284	n.a	

* Guanylurea concentrations; n.a = not available; BCF: bioconcentration factor; BAF: bioaccumulation factor

12 days. This evidence suggests, microbes only used GUA as a nitrogen source, when there is an absence of easily biodegradable carbon.

Finally, Tisler and Zwiener (2019) carried out and study to investigate the formation and biodegradation of GUA and other TPs of MET. From this study, they showed GUA degradation was much faster under anaerobic conditions, whereas MET degradation was fast under aerobic conditions.

4.3. Photodegradation

Quintão et al. (2016) evaluated MET degradation through photolysis (UV-C) and photocatalysis (TiO_2/UV-C). Both methods led to a low degradation of MET, with removal efficiencies of 9.2% for UV-C and 31% for TiO_2/UV-C, after 30 min.

On the other hand, Neamţu et al. (2014) compared the photolytic degradation of eight micropollutants exposed to UV_{254} nm in the presence of H_2O_2 and Fe(II). According to their results, MET was one of the most persistent compounds, with less than 24% of removal, after 60 min of treatment.

4.4. Phytoremediation

Cui and Schröder (2016) assessed the removal efficiency of MET by *Typha latifolia*. After 28 days, the removal efficiency reached a range of 74.0–81.1%. The study concludes that MET can be efficiently removed from aqueous solutions by *Typha latifolia* plants.

Moogouei et al. (2018) evaluated the uptake of MET using arid and semi-arid plants. According to their results, *H. annuus* showed the highest removal efficiency, suggesting that this plant could be considered a potential candidate for phytoremediation of wastewaters in a future.

4.5. Other methods

During their study of photolysis and photocatalysis, Quintão et al. (2016) also assessed the degradation of MET, using an ozonation and chlorination process. In this case, both process showed similar capacities in the depletion of MET, reaching a removal efficiency of 60% after 30 min. However, five by-products were detected, and also were persistent after the treatment.

As a new alternative for the removal of pharmaceuticals in WWTPs effluents, Mohd Amin et al. (2016) tested a combination of clay with biodegradable polymeric flocculants. This clay-starch combination achieved the removal of 70% of the total measured pharmaceutical compounds. However, MET only reach a 21% of removal after 60 min of treatment.

Biological treatments could be efficient for the removal of MET under aerobic conditions, due to its fast degradation. Nonetheless, sequencing batch reactors in WWTPs mostly work under anaerobic and anoxic conditions, which implies a non-quickly and fully degradation of MET. These treatments not only do not remove MET from wastewater, but also produce other TPs, which were also found in high concentrations in multiple water bodies.

Ozonation, chlorination and photodegradation processes do not lead to the complete degradation of MET. These processes should be complemented with other treatment techniques like GO and phytoremediation. We mention these two, because both have reached high removal rates of MET in few time. However, future works should focus trying to demonstrate their efficiency in WWTPs.

5. Toxic effects

The occurrence of MET and its TPs has become a progressively important issue, due to its ubiquitously distribution in the aquatic environment. These contaminants can cause oxidative stress and reproductive toxicity, through endocrine disruption, on non-target organisms (Niemuth and Klaper, 2015; Lee et al., 2019). Due to its high volumes of discharge, its high persistence for degradation and their potential toxic effects on aquatic organisms, it is suggested that MET and its TPs can be become a worldwide threat.

Following sections will discuss the bioaccumulation of MET and its TPs in aquatic animals and plants, as well as the toxic effects on non-target organisms are also discussed. For this purpose, data was summarized in Table 4 and 5.

5.1. Bioconcentration and bioaccumulation

MET and GUA concentrations, as well as bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) for these compounds are summarized in Table 4.

Eggen and Lilo, 2012 investigated the uptake and translocation of MET in edible plant species, and found that MET is accumulated in oily plant tissues. Seeds of rape reached the highest BCF value with 21.72. In comparison, BCFs for grains (cereals wheat, barley an oat) (0.29–1.35), tomato (0.02–0.06), squash (0.12–0.18), bean (0.88) carrot (1.50–3.52) and potato (2.41) were much smaller.

Cui and Schröder, 2016 assessed the uptake and translocation of MET in *Typha latifolia*. Their results showed that MET concentration, in roots, increased the first two weeks of the experiment, until a maximum of 1462.13 $\mu g\ g^{-1}$, but thereafter decreased concentrations. This, could be, due to MET was translocated to other tissues such as rhizomes and leaves.

Regarding aquatic animals, six studies have been carried out in five different species. Le Doujet (2016) exposed juvenile Atlantic salmon to different concentrations of MET during 3, 7 and 10 days. The detected amount of drug measured in carcasses and gills was relatively low compared to the nominal concentration of MET. However, a higher accumulation pattern of MET in gills was observed at the exposure time of 3 days.

De Solla et al. (2016), who measured MET concentrations in caged freshwater mussels, from the Grand River. Although, researchers demonstrated that mussels bioaccumulate other drugs, MET showed no significant uptake or accumulation in mussels.

Meador et al. (2017) studied two fish species from three local estuaries, staghorn sculpin and juvenile Chinook salmon. According to their results, only staghorn sculpin reached a MET concentration above of the reporting limits, with a maximum concentration of 0.028 $\mu g\ g^{-1}$. One year later, they also conducted a laboratory study with 400 juvenile Chinook salmon. Fishes were dosed for 32 days and MET achieved a maximum concentration of 0.039 $\mu g\ g^{-1}$; similar results were reported by Yeh et al. (2017) under the similar conditions.

Ussery et al. (2018) exposed embryonic and larval stage of Japanese medaka for either 24 or 168 h to 10 $\mu g\ L^{-1}$ of MET. Their results, suggest MET that the hardening of the chorion influenced MET uptake and accumulation, since embryos exposed to MET, prior to hardening reported to have higher MET concentrations. Additionally, they also quantified body-burden of MET in larvae, and determined the rate at which larvae medaka can depurate MET after an exposure of 24 h. Body-burden of MET reached a maximum of 3120 $\mu g\ g^{-1}$, and once larvae were transferred to clean water, MET was rapidly excreted, with body-burdens below detection limit within the 24 h.

Table 5
Toxicity produced for metformin and guanylurea.

Species	Metformin Concentrations	Time	Results	Source
Pimephales promelas	40 μg/L	365d	Males: Intersexuality (Frequent PNF and possibly cortical alveolar oocytes clumping throughout testis) Weight ↓	Niemuth and Klaper, 2015
	40 μg/L	28d	Males: VTG mRNA expression ↑	Niemuth et al., 2015
	1, 10, 100 μg/L	7d	Adult: No significant differences Juvenile: VTG mRNA, ERα mRNA, CYP3A126 mRNA, GnRH3 mRNA ↑	Crago et al., 2016
	40 μg/L	365d	AR mRNA, HSD3 mRNA, HSD17β mRNA, CYP19A1 mRNA, and SULT2A1 mRNA ↑	Niemuth and Klaper, 2018
	12.1, 121, 1210, 12100 ng/L	96 h	EOMES mRNA ↑	Johnson, 2018
	12.1, 121, 1210, 12,100	96 h	Neutrophil degranulation no change MPO mRNA no change	Gordon, 2018
Danio rerio	0.1, 1, 10 μg/L	24 h 72 h	KISS1 and KISS1R mRNA ↑ GnRH3 mRNA ↑	Crago et al., 2016 (Unpublished)
	0.01, 0.1, 1, 10, 100 μM	24 h	Mean angle ↑ Maxim accumulated distance ↓	Monshi, 2017
	100, 180, 330, 600, 1100, 1500, 2000 mg/L	96 h	LC_{50} = 1315.5 mg/L	Godoy et al., 2018
	0.05, 0.5, 5, 50, 100, 180, 330, 600 mg/L	120 h	Scoliosis and abnormal pigmentation No significant differences	
Oryzias latipes	20, 40, 80, 160, 320, 640 mg/L	96 h	LC_{50} = 383.3 mg/L	Lee, 2017
	3, 10, 30, 100, 300 mg/L	30d	$NOEC_{(survival)}$ = 100 mg/L	
	0.03, 0.3, 3, 30 mg/L	21d	VTG1 mRNA and VTG2 mRNA ↑ Males: E2, ERα mRNA, ERβ mRNA, VTG1 mRNA, VTG2 mRNA, FSHR mRNA, LHR mRNA, STAR mRNA, CYP11a mRNA, HSD3β mRNA, HSD11β2 mRNA and CYP11b mRNA ↑ T, 11-KT and CYP17 mRNA ↓ Females: 11-KT and CYP11b mRNA ↑ HSD11B2 mRNA ↓	
	10 μg/L	24 h	$ET_{50\ (depuration)}$ = 4.88 h	Ussery et al., 2018
	1, 3.2, 10, 32, 100 μg/L	28d	Stearic acid, palmitic acid, methyl-nicotinamide, and arachidic acid ↑	
	3.2 μg/L	165d	Weight, length, HCD mRNA, HGS mRNA and L-proline ↓ Females: 11-KT ↑	
	* 1, 3.2, 10, 32, 100 ng/L	28d	Weight and length ↓	Ussery et al., 2019
	* 1 ng/L y 7.5 μg/L	165d	No significant differences	
	3.2 μg/L (MET) + 7.5 μg/L (GUA)	165d	No significant differences	
	40, 120, 360 μg/L	4w	Males: CYP19a mRNA, ERα mRNA and ROS ↑ VTG1 mRNA and GSH ↓ Gonad intersex	Lee et al., 2019
	40, 120, 360 μg/L	15w	Females: ERα mRNA and CAT ↑ ERβ1 mRNA and VTG2 mRNA ↓ Spermatogonium-stage cells formation Males: Gonad intersex Females: Spermatogonium-stage cells formation	
Betta splendes	40 μg/L	4w	Males: Time exhibiting gill flaring and tail beats ↓	MacLaren et al., 2018
	80 μg/L	20w		
		4w	Males: Time exhibiting gill flaring and fin spreading ↓	
		20w	Males: Time exhibiting gill flaring and tail beats ↓ Males: Time exhibiting gill flaring and fin spreading ↓	
Salmo trutta fario	1, 10, 100, 1000 μg/L	95d	Liver glycogen ↑	Jacob et al., 2018
	1, 10, 100, 1000 μg/L	108d	Weight ↓ Liver glycogen ↑ Weight ↓	
Limnodynastes peronii	- 0.5, 5, 50, 500 μg/L	30d	Weight, glutamate, leucine, isoleucine, valine, and lactic acid ↑ Length ↓	Melvin et al., 2017
Daphnia magna	* 5–150 mg/L	48 h	$EC_{50\ (immobilization)}$ = 40 mg/L	Markiewicz et al., 2017
	20, 40, 80, 160, 320 mg/L	48 h	$EC_{50\ (immobilization)}$ = 81.4 mg/L	Lee, 2017
	2.5, 5, 10, 20, 40, 80 mg/L	21d	$NOEC_{(survival)}$ = 40 mg/L	
Daphnia similis	5, 8, 12.5, 20, 30, 50 mg/L	48 h	$EC_{50\ (immobilization)}$ = 14.3 mg/L	Godoy et al., 2018
	1, 3, 5, 8, 11 mg/L	14d	$EC_{10\ (reproduction)}$ = 4.4 mg/L	

(*continued on next page*)

Table 5 (*continued*)

Species	Metformin Concentrations	Time	Results	Source
Brachionus calyciflorus	25, 50, 100, 200 μg/L	16d	r ↓	García-García et al., 2017
Plationus patulus	25, 50, 100, 200 μg/L	16d	r ↓	
Mytilus edulis	40 μg/L	7d	VTG mRNA expression ↑ NRRT and V9 mRNA expression ↓ Follicle degeneration and gamete degradation	Koagouw and Ciocan, 2018
Planorbarius corneus	0.01, 0.1, 1, 10 mg/L	35d	Hsp70 ↑	Jacob et al., 2019
	* 0.1, 10, 100 mg/L	21d	Weight ↓ Dilation of the lumen, vacuolization in crypt cells and protrusion of the apex in digestive cells Hsp70 ↑ Weight ↓ Dilation of the lumen, disturb of the compartmentation in the digestive cells, deform of the nuclei, hyperplasia, hypertrophy, and vacuolization in crypt cells	
Lemna minor	6.2, 12.5, 25, 50, 100, 200, 400 mg/L	7d	$EC_{50\ (growth\ inhibition)}$ = 53.7 mg/L	Godoy et al., 2018
Hydra attenuate	2300, 2700, 3100, 3600, 4200, 5000 mg/L	96 h	LC_{50} = 3918 mg/L	
	200, 360, 650, 1200, 2000 mg/L	7d	$EC_{10\ (reproduction)}$ = 701.8 mg/L	
Chlorella vulgaris	1.5, 76.8, 767.8 mg/L	96 h	NPQ ↑ Culture density, ETR, α, Ek and ϕPSII ↓	Cummings et al., 2018

* Guanylurea concentrations; - metformin + bezafibrate + atorvastatin mixture concentrations; ; VTG: vitellogenin; ER: estrogen receptor; GnRH3: gonadotropin-releasing hormone3; KISS1: kisspeptin KISS1R: kisspeptin receptor; E2: 17β-estradiol; FSHR: follicle stimulating hormone receptor; LHR: luteinizing hormone receptor; STAR: steroidogenic acute regulatory protein; HSD3β: 3β-hydroxysteroid dehydrogenase/ delta 5 delta 4-isomerase; HSD11β2: hydroxysteroid 11-β dehydrogenase 2; T: testosterone; 11-KT: 11-ketotestosterone; NPQ: non-photochemical quenching; ETR: electron transport rate; α: electron transport; E_k: minimum saturating irradiance; ϕPSII: effective quantum yield of PSII; PNF: peri nucleolar follicles; ROS: reactive oxygen species; GSH: glutathione; CAT: catalase; NRRT: neutral red retention time; V9: vitelline envelope zona pellucida domain 9; Hsp70: heat shock protein 70; AR: androgen receptor; HSD17β: 17β-hydroxysteroid dehydrogenase; SULT2A1: sulfotransferase family 2A member 1; r: rate of population increase; HCD: β HYDROXYACYL-CoA dehydrogenase; HGS: HMG-CoA synthesis; EOMES: eomesodermin homolog a; MPO: myeloperoxidase.

Finally, Althakafy et al. (2018), quantified six PPCPs in seven invertebrate samples. MET was detected in two different species, may fly and caddis fly. The maximum concentrations of MET found in both invertebrates were 0.312 μg g^{-1} and 0.028 μg g^{-1}, respectively.

5.2. Toxicological effects

In order to discuss the toxic effects produced by MET and GUA in non-target organisms, the reported results were sorted out according to the species under study, as is shown in Table 5.

5.2.1. Fish

5.2.1.1. Pimephales promelas. Niemuth et al. (2015) assessed the effects of MET at environmentally relevant concentrations in fathead minnows. Their results showed MET induced significant up-regulation of VTG in male fish, indicating endocrine disruption. It is thought VTG overexpression may occur as a result of the drug's effects on insulin signaling.

For the same year, Niemuth and Klaper (2015) evaluated male tissues intersexuality, in FHMs exposed to MET. According to their results, exposed FHMs exposed to MET showed a high oocytes occurrence throughout testicular tissue. Suggesting that, MET causes the development of intersex gonads in males, as well as reduce fecundity.

Three years later, Niemuth and Klaper (2018) measured the expression of numerous endocrine-related genes. Their study demonstrated significant up-regulation of the AR, 3β-HSD, 17β-HSD, CYP19A1, and SULT2A1 genes in the testis of FHM exposed to MET.

Finally, Johnson (2018), exposed FHMs to different drugs for 96 h at diverse concentrations in order to identify their effects on T cells. Expression of eomes in the spleen was significantly decreased in FHMs exposed to MET, which may suggest a state of immune suppression, leaving organisms vulnerable to a viral infection.

5.2.1.2. Danio rerio. Crago et al., 2016 reported an increased expression of kisspeptin and kisspeptin receptor at 24 h post fertilization (hpf). Additionally, after 72hpf, expression of GnRH3 was also increased. This relation may be explained, because kisspeptin is vital for the central regulation of GnRh neurosecretory activity and timing puberty.

Monshi (2017) evaluated the behavioral effects of many emerging drugs contaminants (EDCs) in *Danio rerio*. According to their results, MET decreased maximum accumulated distance, and increased the mean angle in a concentration-dependent manner.

Finally, Godoy et al. (2018), reported scoliosis and abnormal pigmentation appeared in embryos exposed to MET, at concentrations of 1100 mg L^{-1}. In addition, they also performed a behavioral assay, where locomotor activity of zebrafish embryos was evaluated. However, researchers reported that the swimming behavior seems not to be disrupted by MET.

5.2.1.3. Oryzias latipes. Lee (2017), investigated the acute and chronic toxicity effects, as well as endocrine disruption effects of MET on this fish. In their study, embryos reached a LC50 of 383.3 mg $L^{-1}$and a survival NOEC of 100 mg L^{-1}. Additionally, male fish exposed to MET showed estrogenic effects, due to an up-regulation on the transcription of the VTG gene transcription.

In an early life stage study (ELS-study), Ussery et al. (2018), demonstrated that fish length and wet weight of larval was significantly decreased by MET. Also, several metabolites associated with cellular energetics as well as with proliferation were significantly altered. One year later, Ussery et al. (2019) carried out a study to characterize GUA toxicity effects on the growth of larval medaka, as well as its persistence into adulthood. Their results showed that fish length and wet weight of larval was significantly decreased by GUA. Growth effects produced by GUA were similar

to those found in medaka exposed to MET. However, these effects occurred at lesser concentrations.

Finally, Lee et al. (2019) evaluated reproductive toxicity and oxidative stress markers in *Oryzias latipes*. According to their results, transcriptions levels of ERα and CYP19a were elevated in male fish, meanwhile on female fish, gene expression of ERβ1 and VTG2 was significantly decreased. Spermatogonium stage cells were observed in female gonads. This suggest MET caused an endocrine disruption in both sex of *O. latipes*.

5.2.1.4. Betta splendens. MacLaren et al. (2018) performed a behavioral study to evaluate aggressiveness of Simase fighting fish after a chronic exposure to MET. After 4 weeks and 20 weeks, an iPad recorded the number and duration of gill flaring, fin spreading and tail beats. Their results showed fish exhibited less aggression toward a dummy male.

5.2.1.5. Salmo trutta fario. To investigate, whether MET impact the gut microbiome, and carbohydrate metabolism of brown trout; Jacob et al. (2018) exposed *Salmo trutta* fario embryos to 5 different concentrations of MET. In general, their results showed that the hepatic glycogen increased in the exposed larvae. Additionally, microbiome analyses indicated an effect of MET on intestinal bacteria, with an increase of Protobacteria and a reduction of Actinobacteria.

5.2.2. Amphibians

5.2.2.1. Limnodynastes peronii. To the extent of our knowledge this is the only study found, that have evaluated MET effects on growth and development in amphibians. Melvin et al. (2017), they exposed *Limnodynastes peronii* tadpoles to a mixture of drugs widely used to treat metabolic syndrome. In their results demonstrated, tadpoles did not show any significant differences in hepatic triglycerides or cholesterol. However, there was an increase, in glutamate, leucine, isoleucine and valine.

5.2.3. Crustaceans

5.2.3.1. Daphnia magna. In order to check if MET, GUA and other six oral antidiabetic drugs might be an ecotoxicological threat, Markiewicz et al., 2017 carried out an acute immobilization test in *Daphnia magna*. At the end of the test, researchers reported an EC50 value of 40 mg L^{-1} for GUA.

As mentioned aboy, Lee (2017) investigated acute and chronic toxicity effects of MET, using Japanese medaka. However, in that study, he also performed an acute and chronic toxicity test with *Daphnia magna*. In this case, after 48 h, embryos reached an EC50 of 81.4 mg L^{-1}. Additionally, survival NOEC was determined at 21d, and got value of 40 mg L^{-1}.

5.2.3.2. Daphnia similis. For *D. similis*, Godoy et al. (2018) performed an acute and chronic toxicity test. In both test, neonates were exposed to different concentrations of MET, for 48 h and 14d, respectively. In acute test, immobile daphnids were recorded, while in chronic exposure, reproduction was assessed. The EC_{50} were 14.3 mg $L^{-1}$and 4.4 mg L^{-1}, respectively.

5.2.4. Rotifers

5.2.4.1. Brachionus calyciflorus & Plationus patulus. These two species of rotifers are widely used for testing the effects of toxicants and xenobiotics. For instance, García-García et al. (2017) quantify the population level changes in these two rotifers exposed to different MET concentrations. After 16d, the population growth of both rotifers was adversely affected by MET.

5.2.5. Mussels

5.2.5.1. Mytilus edulis. As numerous studies suggest, pharmaceutical levels, both in surface water and groundwater, are detected at higher concentrations during dry season. Based on this, Koagouw and Ciocan (2018) studied the cumulative effects of elevated temperature and high concentrations of MET. According to their results, mussels exposed to MET had a decline in neutral red retention time (NRRT), indicating a remarkable lysosomal membrane destabilization. Furthermore, MET caused follicle degeneration and gamete degradation, as well as an upregulation in VTG.

5.2.6. Snails

5.2.6.1. Planorbarius corneus. Jacob et al. (2019) exposed the big Ramshorn snails to multiple concentrations of MET and GUA to assess their impact on the health of gastropod. Stress proteins and lipid peroxides did not show significantly changes. However, in the histopathological analysis, dilatation of the lumen and a disturbed compartmentation of the digestive cells were observed. These observed reactions were only found at the highest concentration of both contaminants.

5.2.7. Aquatic plant

5.2.7.1. Lemna minor. Godoy et al. (2018), exposed *L. minor* plants to multiple concentrations of the antidiabetic drug. After 7 days, growth rates were determined according to the total frond area. The EC_{50} value was of 53.7 mg L^{-1}.

5.2.8. Hydroid

5.2.8.1. Hydra attenuata. Godoy et al. (2018) performed acute and chronic toxicity test in *H. attenuata*. Similarly, *Hydras* were exposed to different concentrations of MET. However, in this case, exposure times were 96 h and 7d, respectively. The LC_{50} and EC_{50} were 3918 mg L^{-1}L and 701.8 mg L^{-1}, respectively.

5.2.9. Algae

5.2.9.1. Chlorella vulgaris. This organism is cosmopolitan and shares similarities in photosynthetic machinery with land plants. Based on this, Cummings et al. (2018) evaluated, whether MET negatively affects chlorophytes photosynthesis. According to their results, non-photochemical quenching (NPQ) value increased over time, suggesting cells were less capable to use the same amount of light energy. Moreover, electron transport rate and minimum irradiance decreased, which also indicate a reduced capacity to process light energy.

Bioconcentration and bioaccumulation factors of MET and GUA suggest both contaminants are barely accumulated in animals. However, in oily plant tissues these contaminants are largely accumulated. It is important to regulate the use wastewater in the irrigation of farmlands, to avoid entrance of MET to the human food web. In general, strategies for assessing bioaccumulation potential of chemicals need to be further optimized and harmonized, as few studies quantify stationary concentrations. MET is well known as an endocrine disruptor, due to the overexpression that produces in many endocrine-related genes. However, the mechanism by which it is generated has not been elucidated.

Studies on early life stage are scarce, as only two authors have reported their results. Embryotoxicity and teratogenic effects of MET and its TPs should be further investigated.

Finally, as MET produced behavioral changes in two different species, *Danio rerio* and *Betta splendens*, it is recommended to study in extent the neurotoxicological effects of this antidiabetic drug.

6. Conclusions, future trends and perspectives

Although in North America and Europe have a huge volume of literature available regarding this topic, both continents still have extremely limited information on the occurrence of MET in oceans and seas. Furthermore, from 2014 to 2019, only Poland reported its MET concentrations in drinking water. Distribution, transport and fate of MET in marine and coastal regions, as well as in drinking water need to be further investigated. On the other hand, most of the countries have showed a lack of interest and necessity for monitoring the occurrence of GUA and other MET TPs. However, all these transformation products should be also monitored. Since MET is accumulated in edible plant species, fish and mussels, this drug could be considered as a potential hazard to the human health. It is necessary to enhance our efforts to remove MET from wastewater and sewage sludge. Further research is needed, in plants; to understand the uptake and translocation processes, and the possible biodegradation pathways of MET on these organisms. In animals, more toxicological studies should be performed to understand the effects of MET and its TPs, because the physiological, biochemical and genetical changes produced in the fish during its exposure to these contaminants are likely to modify the quality of the human food.

Regarding toxic effects, it is important that future works try to unify/harmonize/match their evaluation criteria, because there is an important fluctuation in the evaluation conditions, but also it is important to investigate the effects produced at environmentally relevant concentrations. It should be highlighted the lack of information regarding the toxic effects of GUA and other TPs, this should be urgently further investigated.

Until now, phytoremediation, biodegradation and adsorption have been the best methods to remove MET from water. However, these methods have showed lower removal rates in the field than in the laboratory. It is important to look new alternatives for the removal of this drug in wastewater. For instance, the combination of these methods could be a good option to improve the removal efficiency of MET in WWTPs. Finally as mentioned along this review, MET is highly encountered in the form of GUA in the environment, however there is a huge gap in the study of the removal of GUA from water, this should also be addressed promptly.

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