

Potential and risks of water reuse in Brandenburg (Germany) – an interdisciplinary case study

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ABSTRACT

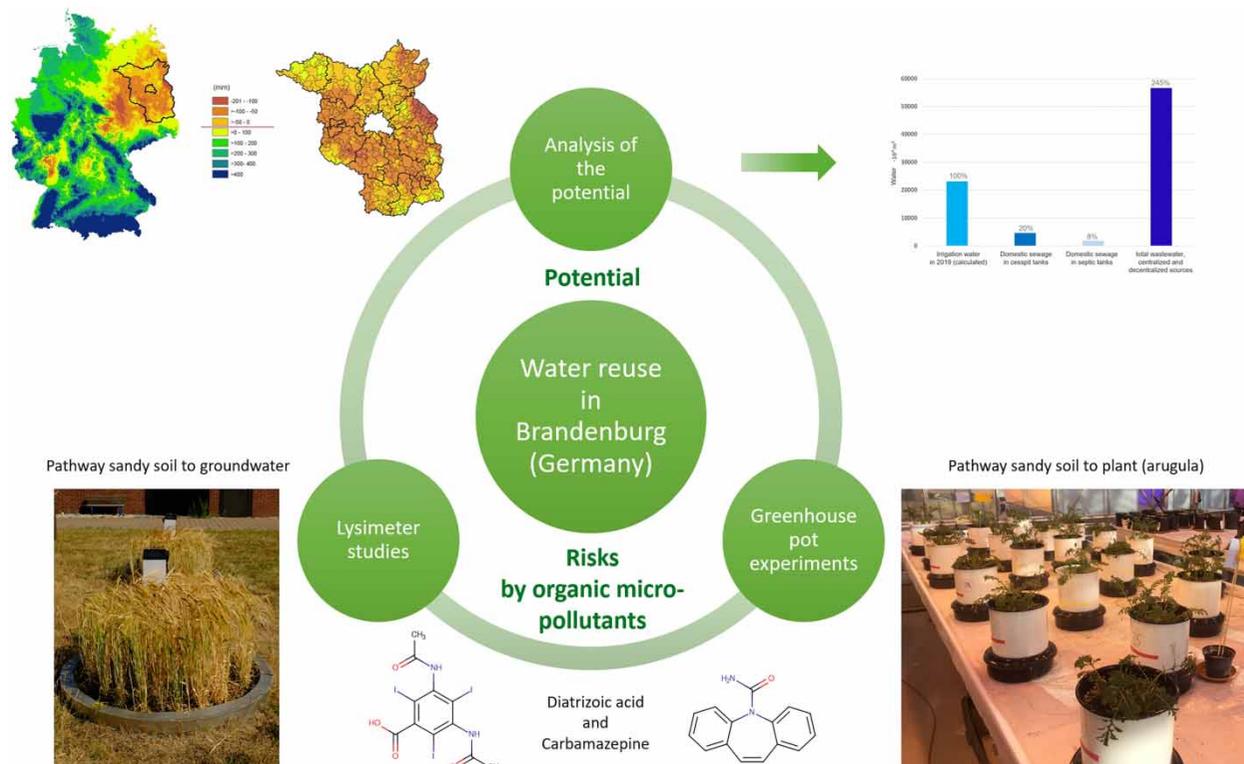
For Brandenburg, a region in Germany with increasing water shortage and drought events, water reuse can counteract competition scenarios between drinking water supply, agricultural irrigation, and industrial use. Centralized and decentralized sources for reclaimed water are found to potentially substitute 245 or 28% of irrigation water, respectively, in agriculture production in Brandenburg. For such a reuse scenario, the fate of organic micro-pollutants is examined for diatrizoate (DZA) and carbamazepine (CBZ). Retention in local sandy soil and transfer into roots and leaves of arugula are analyzed in lysimeter studies and greenhouse pot experiments. Vertical transport was found for DZA and accumulation in or on arugula roots with a root concentration factor of $1,925 \pm 34\%$ but a low bioconcentration factor due to intrinsic molecule properties. CBZ was not found to be mobile in the sandy soil but accumulates in arugula roots and leaves by factors of $70 \pm 7\%$ and $155 \pm 12\%$, respectively. Further research on potential plant uptake and groundwater enrichment for more substances is highly recommended as well as tertiary wastewater treatment prior to water reuse.

Key words: diatrizoic acid, domestic wastewater, emerging contaminants, farmland, organic trace substance

HIGHLIGHTS

- The volume of treated wastewater exceeds irrigation water by a factor of 2.5 in Brandenburg.
- Water reuse could reduce competition between drinking water supply, industry and agriculture.
- Organic micro-pollutant pathways for sandy soil to groundwater and to plants are assessed.
- Carbamazepine did not reach 10 cm depth in lysimeter studies probably due to acidic soil pH.
- Diatrizoate obtained very high concentrations in arugula roots but were low in leaves.

GRAPHICAL ABSTRACT



INTRODUCTION

Exploiting wastewater as irrigation water to counteract water stress is a widely applied option for water reuse in arid regions of the world (Shoushtarian & Negahban-Azar 2020). This is already being implemented extensively, for example in Israel (Reznik *et al.* 2017) or California (Cooley & Phurisamban 2016). The use of reclaimed water (RW) counteracts competition among agriculture irrigation, drinking water supply, and industrial use. Hence, stressed fresh water resources are thought to be protected (Jaramillo & Restrepo 2017; Lavrić *et al.* 2017; Schwaller *et al.* 2021), and benefits in terms of ecological costs can be accounted for (Passarini *et al.* 2014).

Water reuse comes along with potential hazards to human health through pathogen contamination of the irrigation water (Amorós *et al.* 2010; Amahmid *et al.* 2023). However, decades of experience and the development of quantitative risk assessments demonstrated water reuse to be a secure practice (ISO 2020; Wencki *et al.* 2020). If multibarrier principles are applied, which are composed of natural (e.g. reservoirs, soil passage), technical (e.g. filtration, disinfection), and administrative systems (e.g. risk assessment in the catchment, monitoring of connections to the sewage network), microbiological risks can be minimized effectively (Mohr *et al.* 2020).

Anthropogenic trace substances such as pharmaceuticals, pesticides, or industrial chemicals, posing long-term chemical risks, are less regulated (Helmecke *et al.* 2020; Shoushtarian & Negahban-Azar 2020). Most of these organic micro-pollutants (OMPs) are only insufficiently eliminated by wastewater treatment plants (WWTPs) with secondary treatment using activated sludge for reductions of chemical oxygen demand, nitrogen, and phosphorous (Margot *et al.* 2015; Alygizakis *et al.* 2020). Hence, persistent OMPs remain in secondary treated wastewater or irrigation water, respectively, and can accumulate in soil and plant material or contaminate groundwater, posing risks to ecosystems and human health. Dependent on OMPs' intrinsic molecule properties, composition of the soil, cultivated plants, and environmental and climatic conditions of a region, the fate of individual OMPs in environmental compartments is determined (Wu *et al.* 2015). The pathways are mainly divided in transfer of OMPs from soil to groundwater and from soil to plant, thus potentially entering drinking water sources and the food chain.

Ben Mordechay *et al.* (2021) analyzed the concentrations in RW for irrigation, soil, and commercially grown crops for 65 OMPs at 400 fields in Israel and reported that primarily OMPs' concentrations in RW determine the levels detected in soil

and agricultural products. They found that green leaves of RW-irrigated plants obtain higher OMP concentrations than harvest organs of root crops (e.g. carrot and potato) or harvested fruits (e.g. tomato and banana), as also reported in previous studies (Wu *et al.* 2015; Christou *et al.* 2019).

The antiepileptic carbamazepine (CBZ) is a wastewater indicator substance (Jekel *et al.* 2015) and is well studied for plant uptake (Shenker *et al.* 2011; Marsoni *et al.* 2014; Chuang *et al.* 2019). The bioconcentration factor (BCF) and the root concentration factor (RCF) are defined as ratios between OMP concentrations (ng g^{-1}) in plants or roots, respectively, and in soil (McKone & Maddalena 2007) and are reported for CBZ between one and several hundreds. CBZ is, therefore, recommended as a reference for every study on this subject (Wu *et al.* 2015). Furthermore, CBZ can be transformed by soil micro-organisms and plants (Christou *et al.* 2019). The transformation product (TP) 10,11-epoxycarbamazepine accumulates in leaves and is a potentially genotoxic compound with an acceptable daily intake factor of 1,000 lower than that of the parent compound CBZ (Malchi *et al.* 2014; Paz *et al.* 2016). Non-ionic substances have been reported to transfer into plants more easily (Malchi *et al.* 2014), while ionic substances are probably retarded in the phloem (Goldstein *et al.* 2014).

Human biomonitoring studies in Israel (Schapira *et al.* 2020; Ben Mordechay *et al.* 2022) revealed elevated CBZ concentrations in urine of men, women, and children, most of them being vegetarians consuming vegetables irrigated with RW. Schapira *et al.* (2020) pointed out the need to resolve the contradiction between recommended daily vegetable consumption and the chemical risks of RW irrigation.

Risk assessments often determine the thresholds of toxicological concern (TTC) with differing results depending on OMP concentrations and irrigated crops. On the one hand, negligible health risks are reported for CBZ ($\text{BCF} > 100$) in maize, rice, ryegrass, and wheat (Delli Compagni *et al.* 2020) as well as for diclofenac ($\text{BCF} > 100$ after 3 years) in tomatoes through uptake from RW after tertiary treatment and disinfection (Christou *et al.* 2017). On the other hand, comparable low CBZ concentrations ($0.5 \mu\text{g L}^{-1}$) lead to elevated 10,11-epoxycarbamazepine concentrations in the leaves of carrots and sweet potatoes. The TTC value of 62.5 ng kg^{-1} for children of 25 kg was found to be exceeded by eating 25 g of carrot leaves or 90 g of sweet potato leaves per day, which is common in parts of Asia and Africa. This does not imply direct toxic effects but indicates the need for detailed toxicity analysis on this OMP. Moreover, there are other OMPs, e.g. lamotrigine with a TTC value of 2.5 ng kg^{-1} for children that is exceeded by eating half a carrot (60 g) per day (Malchi *et al.* 2014).

Previous studies conclude that more research is highly recommended, regarding different agricultural products, substances, soils, and climatic conditions to properly manage related risks (Helmecke Fries & Schulte 2020). The European regulation on minimum requirements for water reuse is applied since June 2023 (EU 2020) and is in line with worldwide regulations and guidelines on water reuse providing no restriction for OMPs in spite of recommending their consideration in the site-specific risk management (Shoushtarian & Negahban-Azar 2020).

The current study, therefore, focuses on a sandy soil typical for the state of Brandenburg. This is one of the driest regions in Germany, with an annual mean rainfall of 580 mm and extreme droughts in recent summers. The annual climatic water balance (Figure 1) is negative with -52 mm by a mean temperature of $9.7 \text{ }^\circ\text{C}$ ($1.4 \text{ }^\circ\text{C}$ in winter, $18.4 \text{ }^\circ\text{C}$ in summer). In order to investigate whether water reuse is a viable option to mitigate competition for water in our case study region, we studied

- (a) potential sources for RW in Brandenburg and irrigation demand,
- (b) the transfer pathway soil to groundwater for the iodinated X-ray contrast agent diatrizoate (DZA) that is comparably large in size and CBZ (as indicator OMP) in disturbed soil lysimeters, and
- (c) the transfer pathway soil to plant also for DZA and CBZ in greenhouse pot experiments with arugula.

The obtained interdisciplinary perspectives are interconnected through the same sandy soil and RW used for irrigation, which allows for common evaluation of separately analyzed pathways soil to groundwater and soil to plant. Since the focus is first on potential water sources and second on the risks of OMP transfers, agricultural yields are not assessed and no nutrient deficiency experiments are conducted.

MATERIALS AND METHODS

Analysis of the potential of water reuse for Brandenburg

Wastewater generated in Brandenburg is classified in centralized (sewage network and WWTP) and decentralized disposal, subdivided in cesspit tanks and septic tanks (small-scale WWTP). Respective amounts are officially reported for 2019 (MLUK 2021) and a mean water consumption of 106 L d^{-1} and person (Destatis 2018) was used for calculations. The actual use of

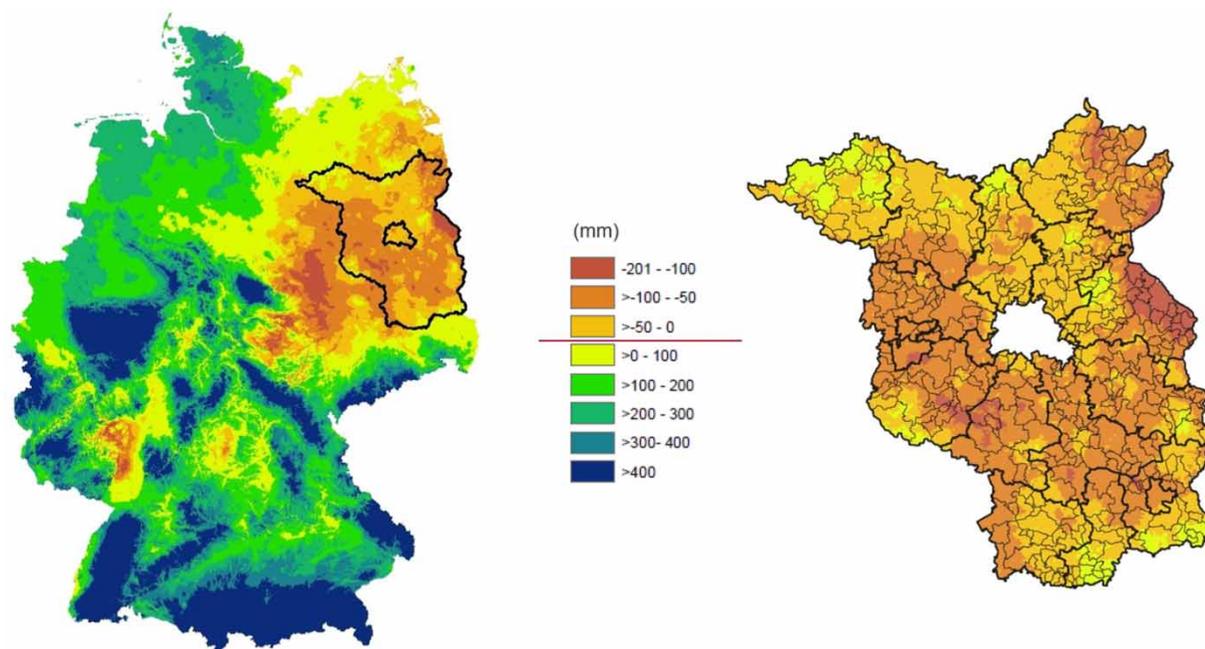


Figure 1 | Annual climatic water balance of Germany (left) and the state of Brandenburg (right) over the period from 1991 to 2021. Colors indicate the local water balances, calculated from precipitation minus potential evapotranspiration. Based on data from the German Climate Data Center (CDC 2023).

irrigation water was reported lastly for 2009 (Berlin-Brandenburg 2012) and, therefore, needed to be projected based on the agriculturally used area in 2019 (Destatis 2021). Calculations were done for the growing season (April–October) and for the whole reference year 2019.

Furthermore, fertilizer application was analyzed for potential substitution by remaining nitrogen (N) and phosphorous (P) in RW. Due to lack of available data, the mean value of domestic sales volume in 2019–2021 was used to estimate applied fertilizer amounts (Destatis 2018). Human excreta are calculated with 11 g N and 1.8 g P contents per day and person.

Reclaimed water and soil characteristics

RW was taken from the effluent of a large WWTP in Berlin with secondary treatment and UV-disinfection between May 1 and September 30. Each batch was analyzed for OMP concentrations immediately after collection from the WWTP, and the stored RW was reanalyzed to evaluate potential OMP losses. Accompanying chemical parameters for RW are summarized in Table 1.

The sandy soil was taken from an agricultural field in Brandenburg (north east Germany, 52°16'34.3" N 13°04'39.7" E), which is located in a region that was formed in the last ice age as a terminal moraine. The soil material was taken from four different layers (Table 2), with layer A representing the top soil of horizon A and all subsequent layers representing the mineral horizon.

Table 1 | Characteristic parameters for RW used for irrigation

	Base parameters		Anions				Cations			
	pH	EC $\mu\text{S cm}^{-1}$	NO_3^- mg L^{-1}	PO_4^{3-}	Cl^-	SO_4^{2-}	K^+ mg L^{-1}	Na^+	Ca^{2+}	Mg^{2+}
<i>n</i>	5	5	11	15	3	6	15	15	15	15
Mean	7.5	1,212	37.8	0.59	155.4	110.6	84.9	28.5	11.3	112.7
SD	0.2	66	2.9	0.21	7.7	7.9	16.0	1.9	1.2	7.3

Notes: *n* is the number of measurements of RW batches, EC is the electrical conductivity representing the salinity of RW, and SD is the according standard deviation.

Table 2 | Physico-chemical properties of the four layers of sandy soil

Layer	Depth cm	Texture class	Textural fractions (%) ^a			Bulk density g cm ⁻³	C _{org} %	pH _{H2O}	pH _{CaCl2}
			Sand	Silt	Clay				
A ^b	0–30	loamy Sand	83.3	11.5	5.3	1.53	0.73	5.4	4.9
L1	31–46	loamy Sand	87.4	7.9	4.7	1.72	0.16	5.7	5.4
L2	47–80	loamy Sand	89.6	7.0	3.4	1.70	0.15	5.8	5.4
L3	81–100	sandy Loam	71.4	17.2	11.5	1.75	0.07	6.4	5.9

Notes: C_{org} is the organic carbon content in wt.%. pH values measured in ultrapure water and 0.01 mol L⁻¹ CaCl₂ with a soil solution ratio of 1:2.5.

^aAnalyzed by the PARIO method (Durner & Iden 2021).

^bDetermined for pot experiments: 0.57% C_{org}; 0.65% C_t; 0.06% N_t; and 4.98 cmol_c kg⁻¹ CEC_{pot}.

Lysimeter studies

Two lysimeters (1 m² cross-sectional area and 1 m depth) were filled with sandy soil according to Table 2 and planted with summer barley during the vegetation periods 2021 (May 26–August 25) and 2022 (March 7–July 14) and lay fallow in the interim. Mineral fertilizer was applied in two applications during each vegetation period to optimally supply plants with nutrients. Accordingly, 8, 2.6, and 10 g m⁻² of N, P, and K was applied in 2021, and 8.1, 1.3, and 5 g m⁻² N, P, and K in 2022, respectively. Therefore, no effect of fertilization by the RW was considered in the experiments. All cumulated fluxes at the upper boundary during the total experimental period (May 26 2021–November 1 2022) of the lysimeters are displayed in Figure 2. The total inflow of 1,594 L m⁻² is comprised of 850 L m⁻² precipitation, 543 L m⁻² RW irrigation, and 200 L m⁻² tap water irrigation. Precipitation was measured by a near tipping bucket corrected after Richter (1995). The potential evapotranspiration accounted for 1,500 L m⁻². During the vegetation period, RW and tap water irrigation were demand-based. In addition, groundwater recharge was simulated by irrigation with RW along the winters 2021/22 and 2022/23. Hence, seepage water could be collected all over the year. Each lysimeter is equipped with nine glass suction cups (ecoTech Umwelt-Messsysteme GmbH, Bonn, Germany), inserted every 10 cm. One to four times a month, the seepage water was sampled by applying a suction of 400 hPa for at least 4 h on all suction cups. Furthermore, samples were collected from a suction array at 100 cm depth (constant suction of 70 hPa), and of the free draining water at the lower boundary passing the suction array, which were sampled regularly depending on the quantity accrued (twice a week to once a month). Mass balances for OMPs are estimated by the volume and concentration of the influent (irrigation) and the effluent (suction array and free drainage water).

Greenhouse pot experiments

Arugula (*Eruca sativa* var. 'Speedy') was cultivated in the greenhouse (artificial light for 12 h d⁻¹, mean temperature 18 ± 2 °C, mean relative humidity 49 ± 13%) by using standard 'Mitscherlich pots' (max. vol. 6,200 mL, diameter 20 cm, height 21 cm, see Figure 3, right), five plants per pot, each filled with 6,500 g sieved (2 mm) and homogenized air-dry agricultural topsoil (see Table 2, Layer A). All pots were regularly watered to a maximum field capacity of 84%, using the RW, with three replicates. Arugula was watered by simulating drip irrigation, where leafy parts of the arugula did not have any contact with the irrigation water (in comparison to overhead or sprinkler irrigation). To avoid unwanted growth depression, an initial fertilization was given to all pots (Table 3).

Analyzed arugula was harvested after 65 days at BBCH¹ stage 59. Harvested plant material was dried at 30 °C and homogenized afterward with a ceramic ball mill for further analyses. Soil samples were taken from each vegetated pot immediately after harvest and were air dried.

Extraction of samples of reclaimed water, soil, and plants

The water samples were stored at -20 °C until sample preparation, then centrifuged for 10 min at 5,000 rpm at room temperature and for an additional 10 min at 13,000 rpm at 4 °C, and subsequently analyzed by direct injection.

¹ Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie.

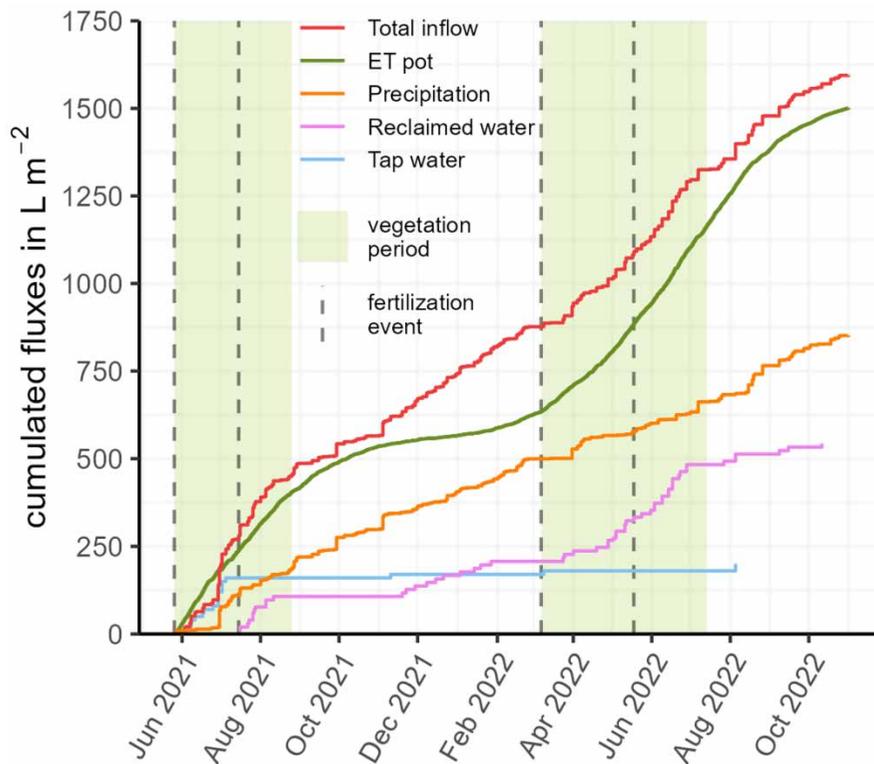


Figure 2 | Cumulated upper boundary fluxes of lysimeters during the experimental period. The outflowing flux potential evapotranspiration (ET pot) is given in absolute values for better comparison with the inflowing fluxes.



Figure 3 | Lysimeters with summer barley (left) and pot experiments with arugula in the greenhouse (right).

The solid-liquid extraction for soil and plant samples was adapted from a method by [Riemenschneider *et al.* \(2017\)](#). The roots and leaves of the arugula plant were processed individually by weighing 0.5 g of soil or plant material in a 15 mL centrifuge glass. Extraction was performed twice using a mixture of methanol and Milli-Q water (1:1, v:v). Following the protocol of [Riemenschneider *et al.* \(2017\)](#), an aliquot of the two combined supernatants of both extractions were centrifuged for 10 min at 13,000 rpm and 4 °C.

Table 3 | Initial nutrient application of the pot experiments

Nutrient	mg kg ⁻¹ dry soil	Applied as
N	400	Ca(NO ₃) ₂ · 4 H ₂ O
P	119	KH ₂ PO ₄
K	150	
Mg	60	MgSO ₄ · 7 H ₂ O

Analysis and quantification

In lysimeter studies, OMPs were analyzed by high-performance liquid chromatography (HPLC) coupled to a triple-quadrupole mass spectrometer (MS/MS) as described in more detail by Zeeshan *et al.* (2023). The analyzed OMPs were quantified using an internal calibration with isotopic labeled standards.

OMP analyses for the pot experiments were performed by using supercritical fluid chromatography (SFC) (Waters Acquity UPC2 system) coupled to a triple-quadrupole mass spectrometer (Waters TQXS) using a Ethylene Bridged Hybrid (BEH) column based on a method by Schulze *et al.* (2020). For quantification of CBZ and DZA in RW samples, external calibration was used. The apparent recoveries were included by spiking the samples with the reference standards. For soil and crop samples, matrix-matched calibrations using non-treated soil and arugula material were used. In addition, for soil samples, apparent recoveries were included analogous to the water samples.

RESULTS AND DISCUSSION

Potential of water reuse for Brandenburg

About 2.5 million people live in the state of Brandenburg (Germany) on an area of 29,650 km². About 46% of the area is used for agriculture while about 37% is covered by forest. As shown in Figure 1, large regions have a negative climatic water balance, additionally suffering droughts in summer. Water irrigated demand for agriculture in Brandenburg was calculated to be $23.1 \times 10^6 \text{ m}^3$ in 2019 (average 72.2 mm) while about $56.6 \times 10^6 \text{ m}^3$ total wastewater is generated in the growing season from April to October (Figure 4). This wastewater could potentially be reclaimed and could substitute irrigation water withdrawn from surface and groundwater.

About 11% of Brandenburg's inhabitants are not connected to sewage networks. A total of 75,000 use septic tanks for wastewater disposal and 205,000 use cesspit tanks for wastewater storage. Vacuum trucks are regularly collecting the stored wastewater and transport it several kilometers to the next sewage network connection. These decentralized sources for RW alone could theoretically supply one-fourth (28%) of irrigation water needs in 2019 (Figure 4). This may offer potential for innovative point-of-use reuse concepts fitting to local socio-economic structures (UBA 2021).

Furthermore, RW add loads of nutrients to the field and can therefore substitute parts of fertilization. We estimate that about 68,500 t N (as ammonia) and 3,400 t P (as phosphorus pentoxide) are applied to the fields in Brandenburg each year. With regard to the untreated wastewater in cesspit tanks, amounts of 4,300 t N and 1,000 t P accrue during a growing season, which could substitute theoretically a maximum of 6% N fertilizer and 28% P fertilizer. With respect to much lower concentrations in the effluent of WWTPs (exemplarily calculated with data from Table 1), only 145 t N and 150 t P with substitution potential of 0.2% N fertilizer and 4.4% P fertilizer, respectively, would have been reached by replacement of the irrigation water with RW in 2019.

The calculated (maximum) potentials for irrigation water substitution appear to be promising. The concomitant fertilizer substitution potentials by dissolved nutrients in RW are a positive side effect and worth considering in fertilization planning. At the same time, negative effects on plants could arise from salts contained in RW (Ofori *et al.* 2021). An electrical conductivity (EC) of $1,212 \mu\text{S cm}^{-1}$ found in the analyzed RW (cf. Table 1) indicates a slight to moderate restriction for salt-sensitive plants in arid or semi-arid regions (Ayers & Westcot 1985). However, this does not apply for our case study region and, therefore, EC is not restricted. The perspective of irrigation water demand and available RW sources indicates water reuse as a viable option for Brandenburg to counteract water scarcity and competition. Putting this into practice would require further consideration of infrastructure, transportation, and water treatment, especially for the decentralized sources. However, risks by dissolved contaminants (OMPs) need to be assessed with another perspective.

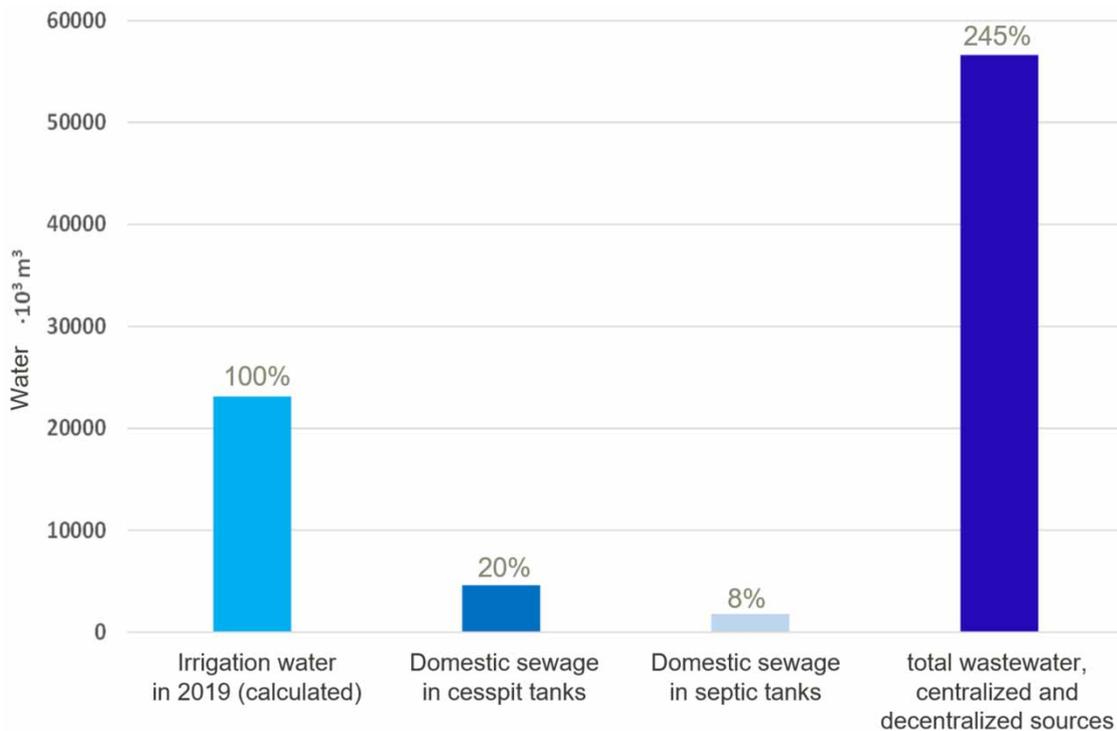


Figure 4 | Water balance during the growing season (April–October). Accounted for substitution potential for irrigation water used in 2019 with decentralized sewage sources and total wastewater in Brandenburg, Germany.

Pathway soil to groundwater – lysimeter studies

To study potential transfer of OMPs into groundwater, two lysimeters were exemplarily evaluated for DZA and CBZ over a 16-month period (July 2021–October 2022). The temporal concentration courses in different depths were similar for both lysimeters (Figure 5).

DZA and CBZ concentrations in the used RW batches for irrigation ranged between 0.6 and 3.3 $\mu\text{g L}^{-1}$ and 0.3 and 1.4 $\mu\text{g L}^{-1}$, respectively (Figure 5, top ‘RW’). After the vegetation period of 2021, the seepage rates increased and DZA was successively transported down the soil profile, clearly visible by the temporally shifted concentration peaks along the lysimeter depths (Figure 5, $z = 10\text{--}90\text{ cm}$). The concentration decreases with increasing soil depth due to diffusion and hydrodynamic dispersion. Five months after the start of RW irrigation, DZA reaches the lower boundary (i.e. the effluent) of the lysimeters (Figure 5, bottom). The applied DZA mass, from July to August 2021, has left the lysimeters at the lower boundary until April 2022. These observations suggest that neither sorption nor degradation play an important role for the fate of DZA in sandy soils. These findings are supported by our observations in laboratory batch experiments, where neither degradation nor sorption was observed (data not shown). Moreover, a similar behavior of DZA was found in other studies. Ternes *et al.* (2007), for example, observed a very persistent and mobile behavior for DZA in lysimeters also filled with a sandy soil and suggested that DZA could be used as conservative wastewater tracer in soils and groundwater. Kalsch (1999) and Haiß & Kümmerer (2006) investigated biodegradability of DZA in activated sludge and also found that DZA is persistent.

Interestingly, the DZA concentration peaks in the upper layers (up to 40 cm) during the vegetation period in 2022 are roughly two times higher than that in RW concentrations. This phenomenon is explained by high evapotranspiration in the vegetation period (Figure 5, green area in the top subplot ‘RW’), when water leaves the system due to vaporization of root water uptake and DZA remains in the soil. In particular, the increased concentration in 40 cm depth suggests that DZA is not or only partly taken up by the barley plants.

Generally, CBZ concentrations were very low in all depths (Figure 5, $z = 10\text{--}90\text{ cm}$). Solely in 10 cm depth, values above the limit of quantification (LOQ) of 5 ng L^{-1} were detected during the irrigation periods, due to macropore transport. These findings suggest that CBZ is either strongly sorbed to the mineral or organic surfaces and/or microbially transformed within

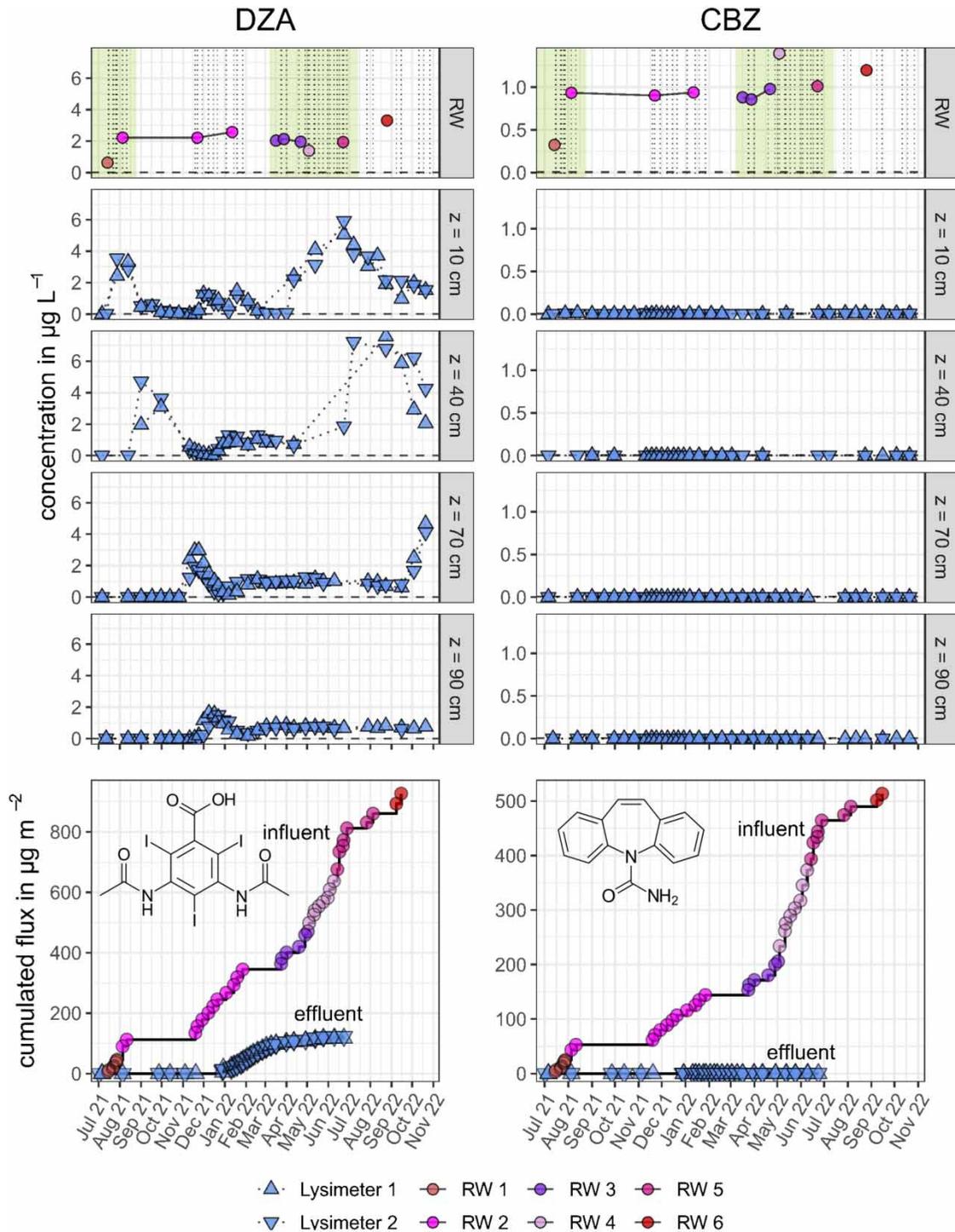


Figure 5 | Top row: concentrations of DZA and CBZ in the RW batches (first subplot) used for irrigation; rows 2–5: concentrations in the suction cup samples of four selected depths. Each vertically dotted line in the ‘RW’ subplot equals an irrigation event of 10 L. The green area marks the vegetation period of summer barley. Bottom: total cumulated flux in (influent) and out (effluent) of the lysimeters.

the first few centimeters of the soil. Hence, none of the CBZ applied with the irrigation water reached the effluent. This assumption is consistent with conducted laboratory batch experiments under aerobic conditions, in which especially sorption reduced the concentration of CBZ in the soil solution (data not shown). Several other studies reported minor biodegradability for CBZ under aerobic condition classifying it as a persistent substance in the vadose soil zone (Li *et al.* 2013; Grossberger

et al. 2014; Thelusmond *et al.* 2018); however, König *et al.* (2016) reported anaerobic transformation of CBZ during bank filtration. Ternes *et al.* (2007) observed that sorption and/or biodegradation took place to a certain extent during the passage of CBZ through lysimeters. However, in their experiments, degradation/retardation of CBZ during the soil passage was relatively low and CBZ was detected in all sampled depths of their lysimeters. Similar to the findings of Ternes *et al.* (2007), Paz *et al.* (2016) also detected CBZ in all depths of their lysimeters (1 m depth), one filled with a sandy loam and another with loamy sand soil. CBZ sorption is known to be mainly governed by soil organic matter (Paz *et al.* 2016), whose quality might change at different pH values (e.g. due to protonation and deprotonation of functional groups). Thus, the contradictive findings may be explained by soil pH, since Ternes *et al.* (2007) and Paz *et al.* (2016) reported neutral pH values and our examinations show slightly acidic soil pH (Table 2). Accordingly, the environmental fate of CBZ can differ strongly due to site-specific effects related to soil properties.

To this state, no threshold concentrations or maximum loadings exist for OMPs regarding the soil to groundwater transfer pathways in German legislation². However, the regulatory aspects of RW for irrigation are frequently discussed, and environmental and health quality criteria with regard to OMP ought to be developed (Helmecke *et al.* 2020).

Pathway soil to plant – greenhouse pot experiments

To study plant uptake after irrigation with RW, pot experiments were performed in a greenhouse using arugula as a model plant. DZA and CBZ concentrations were quantified in RW, soil, arugula roots, and leaves (Figure 6). Both DZA and CBZ could be detected in every compartment above their LOQ. Concentrations in RW were fluctuating slightly around 1.5 (DZA) and 1 $\mu\text{g L}^{-1}$ (CBZ) and are comparable to the data shown in the lysimeter studies above (Figure 5), since the same source of RW was used.

DZA was found in highest concentrations in arugula roots between 2,400 and 6,700 ng g^{-1} dry weight (d.w.), whereas much lower concentrations of around 70 ng g^{-1} d.w. were quantified in arugula leaves. This is also shown by the calculated root and BCFs, demonstrating that DZA is taken up by the roots but not transported to the leaves (Table 4). However, as discussed elsewhere (Castan *et al.* 2023), anionic chemicals like DZA are only partially absorbed due to the negatively charged cell membranes of the roots and the resulting repulsive forces. This implies that DZA may not pass through the root epidermis and is only attached to the root surface. In contrast, concentrations in the soil material were significantly lower around 2 ng g^{-1} d.w. This demonstrates the poor sorption potential to soil surfaces as well as the mobile character of DZA transporting the compound with the water toward the plant. These data are in line with the results from the lysimeter studies above, showing that DZA tends to be strongly transferred vertically with the water and can potentially reach groundwater (cf. Figure 5).

Another OMP analyzed was CBZ, which is well studied and can thus act as an indicator substance. CBZ was identified in low concentrations in the soil material (<1 ng g^{-1} d.w.). As reported elsewhere (Scheytt *et al.* 2006; Martínez-Hernández

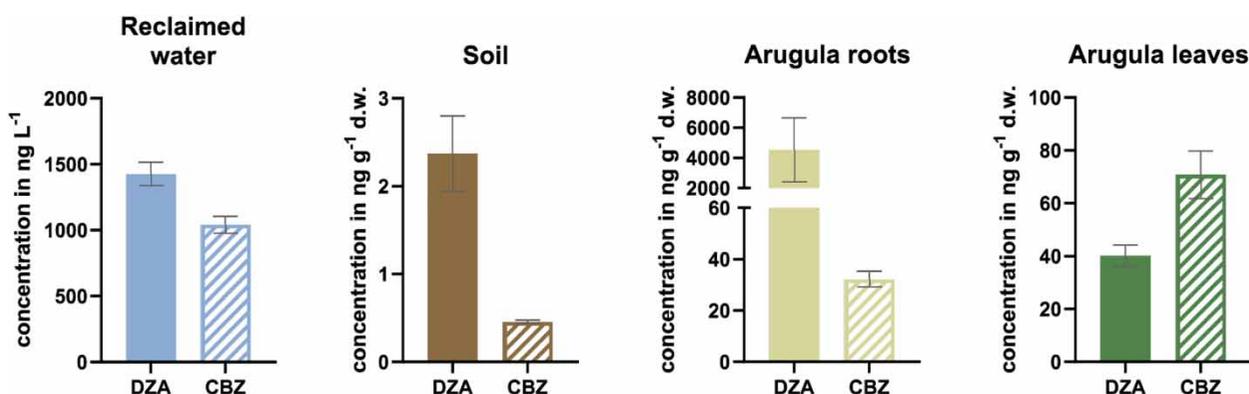


Figure 6 | Concentrations of CBZ and DZA in RW used for irrigation and in soil and plants, separated in arugula roots and leaves, referred to d.w., after harvest.

² Federal Soil Protection and Contaminated Sites Ordinance of Germany from 1999.

Table 4 | Mean values of BCFs and RCFs of DZA and CBZ referred to the soil material irrigated with RW

	DZA	CBZ
BCF [-]	18 ± 4	155 ± 19
RCF [-]	1,925 ± 664	70 ± 5

et al. 2016), sorption of CBZ is low. These results stand in contrast to those shown in lysimeter studies above since a removal of CBZ in the very upper soil layers was shown. This can result from a removal of CBZ by biodegradation in soil and plant material, e.g. TPs of CBZ were reported in several crops (Malchi *et al.* 2014; Martínez-Hernández *et al.* 2016; Riemenschneider *et al.* 2016). In addition, it is shown that through the uptake of RW, increased concentrations of CBZ were found in both plant materials in similar levels at around 71 (arugula leaves) and 32 ng g⁻¹ d.w. (arugula roots). Goldstein *et al.* (2014) reported comparable results for plant uptake of CBZ irrigated with treated wastewater containing similar concentrations of CBZ. Also, the RCF (70) and BCF (155) for CBZ show an uptake by arugula.

In comparison, these two chemicals showed different uptake capacities by the plant. While for DZA a BCF of 18 (less uptake) was calculated, CBZ has a much higher potential for plant uptake (BCF = 155). This can possibly result from its ionic state, since non-charged molecules, like CBZ (pH 7.5), may more readily cross cell membranes.

In addition, the maximum amounts of fresh arugula that would still be tolerated from a health perspective for the determined concentrations of DZA and CBZ were calculated. Since both DZA and CBZ are classified as Cramer class III (Malchi *et al.* 2014; Neumann & Schliebner 2019), a TTC value of 1.5 µg/kg bodyweight and per day was used (Kroes *et al.* 2004). For DZA ($c_{f.w.} = 6.68 \text{ ng g}^{-1}$), an adult with 70 kg bodyweight could eat 16 kg of arugula per day, while for CBZ ($c_{f.w.} = 11.80 \text{ ng g}^{-1}$), 9 kg arugula per day could be eaten to remain below the threshold. These values are comparable to the ones from Malchi *et al.* (2014). The amounts stand in no relation to the daily amount of fresh arugula a person normally consumes and thus are negligible for an adult from a single substance perspective.

CONCLUSIONS

In this study, we showed interdisciplinary examinations on water reuse providing perspectives that are necessary to elucidate potentials and risks. Our case study region, Brandenburg (Germany), possesses centralized and decentralized sources for RW. Effluent from centralized WWTPs could substitute 245% while decentralized cesspit and septic tanks could substitute 28% of water used for agricultural irrigation in 2019. Furthermore, fertilizers could partly be substituted by nutrients in RW from cesspit tanks, and storage options during winter time may increase the potential. Water reuse is, therefore, a viable option to mitigate competition for water in Brandenburg. However, implementation requires further consideration of infrastructure, transportation, and water treatment, especially for decentralized sources. As a limitation, it must also be considered that minimum discharge from WWTPs may be a requirement for streams.

The perspectives of OMP transfer pathways from local sandy soil to groundwater and from the soil to plant were examined by lysimeter studies and greenhouse pot experiments. The fate of the comparable large and heavy X-ray contrast media DZA and the antiepileptic CBZ as common indicators for OMP was analyzed. This should allow for a first risk estimation based on realistic OMP concentrations in the RW.

DZA showed tracer-like transport in the lysimeters without retardation posing a high risk for groundwater contamination. Also, DZA was found to accumulate in or on the roots of arugula with a remarkable RCF of $1,925 \pm 34\%$ in pot experiments. Probably it is transported with the water flux due to its high mobility but is not able to pass through the epidermis due to its size and negative charge. However, a small part of DZA was quantified in arugula leaves with a BCF of $18 \pm 22\%$.

CBZ seems to be removed in the very upper soil layers in the lysimeter studies. It, therefore, poses no risk for groundwater contamination for the sandy soil of our case study. However, other studies showed vertical CBZ transfer in sandy soil that potentially indicates a long-term risk also for groundwater in Brandenburg. Contradicting, CBZ could not be found in greater concentrations in the soil material of the pot experiments demonstrating (a) no or barely any sorption to it or (b) probably transformation. However, CBZ showed an uptake by arugula with $70 \pm 7\%$ RCF and $155 \pm 12\%$ BCF indicating a potential transfer toward plants.

While bringing together these perspectives, water reuse with RW can be a promising option for agricultural irrigation in Brandenburg, if water quality is managed with regard to OMP and potential transfer pathways. The two examples, DZA

and CBZ, showed a wide range of possible environmental fates and related risks due to substance-specific properties. Comparison with literature data showed that soil properties (inorganic, organic, and microbiological) together with cultivated plants alter OMPs' fate.

For a sound risk management, fate predicting tools for wastewater treatment, the pathway soil to groundwater, and the pathway soil to plant would be very helpful. As this has to be based on intrinsic molecule properties, local soil properties, and agricultural product, this is a major challenge, especially since there is a lack of general parameters (e.g. D_{OW} or D_{OC}) to predict sorption of potentially ionic OMPs (Sigmund *et al.* 2022).

However, the load of OMPs in RW is the driving parameter for concentrations found in the environment. It is, therefore, highly recommended to implement advanced wastewater treatment (tertiary treatment). In addition, treatment should be selected based on the case-specific OMPs, which are considered most likely to contaminate groundwater and plants in the area where the water is to be reused.

As shown for DZA and CBZ, polar and mobile as well as non-polar and non-mobile OMPs are transferred into agricultural products. Hence, to address all these aspects, monitoring and extended analyses of OMPs are needed to minimize potential risks.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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