



Research article

Do dietary exposures to multi-class endocrine disrupting chemicals translate into health risks for Gangetic dolphins? An assessment and way forward



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ABSTRACT

Dietary exposure risks of 39 multi-class Endocrine Disrupting Chemicals (EDCs) to the threatened Gangetic dolphins (*Platanista gangetica*) were investigated in a conservation-priority segment of the Ganga River. Elevated EDCs bioaccumulation was observed across prey fish species, with di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) significantly contributing to the EDC burden. The concentrations of persistent organochlorines in prey revealed a shift from dioxin-like polychlorinated biphenyls (PCBs) to non-dioxin-like PCBs. The prevalence of regulated p,p' DDT (Dichlorodiphenyltrichloroethane) and γ -HCH (Lindane) residues suggests regional non-compliance with regulatory standards. The concentration of some EDCs is dependent on the habitat, foraging behavior, trophic level and fish growth. The potential drivers of EDCs contamination in catchment includes agriculture, vehicular emissions, poor solid waste management, textile industry, and high tourist influx. Risk quotients (RQs) based on toxicity reference value were generally below 1, while the RQ derived from the reference dose highlighted a high risk to Gangetic dolphins from DEHP, DDT, DnBP, arsenic, PCBs, mercury, and cadmium, emphasizing the need for their prioritization within monitoring programs. The study also proposes a monitoring framework to provide guidance on monitoring and assessment of chemical contamination in Gangetic dolphin and habitats.

1. Introduction

The probable extinction of the Yangtze River Dolphin, a tragedy propelled by a myriad of human-induced pressures, starkly illustrates the precarious situation of riverine dolphins across the globe [1]. With only five extant species of river dolphins worldwide, all classified as threatened, the imperative to address their vulnerability to various anthropogenic stressors is more urgent now than ever [2]. These vulnerabilities are particularly pronounced for species in densely populated river basins undergoing rapid urban development, such as the Gangetic Dolphin (*Platanista gangetica*) in the Ganga Basin [2].

As an apex predator, Gangetic Dolphin (henceforth referred to as GD) plays a key role in maintaining the structural and functional integrity of freshwater ecosystems through top-down processes. The GD is often considered an appropriate umbrella species, offering protection not only for itself but also facilitating conservation efforts for other co-occurring species [3–5]. This makes understanding

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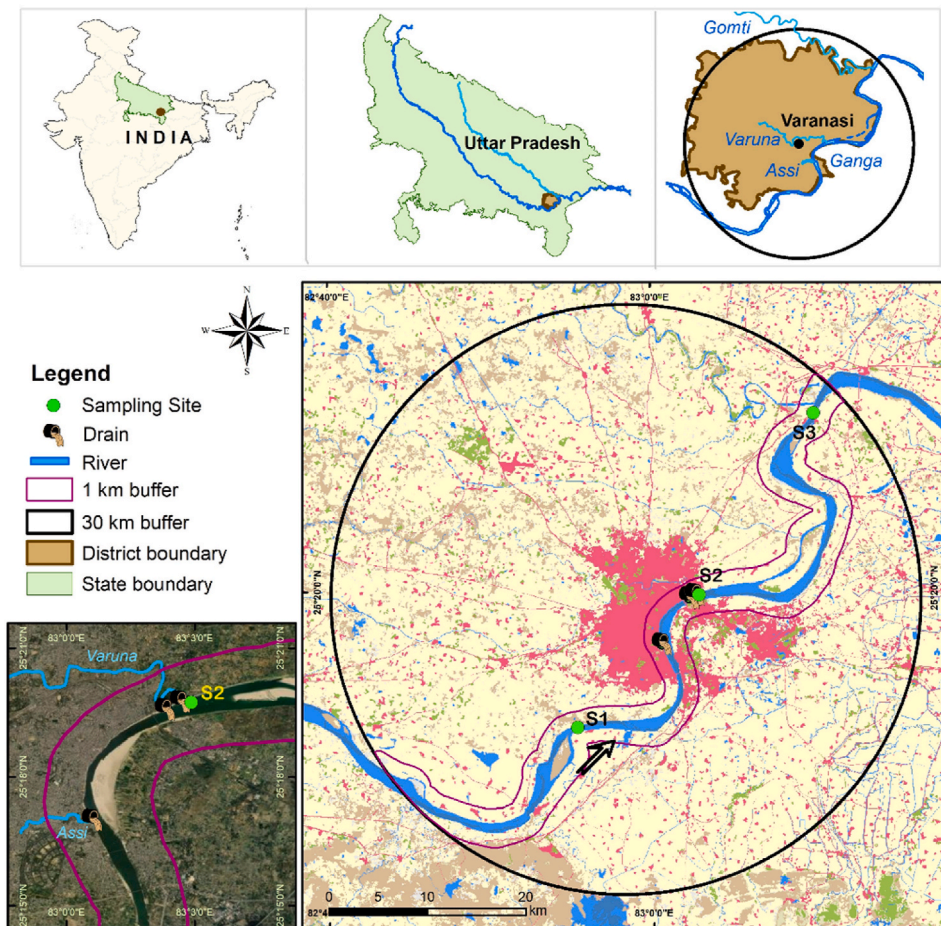


Fig. 1. Representation of sampling sites along MGR.

GD's responses to various anthropogenic stressors pivotal for devising effective conservation and management strategies that benefit the entire freshwater ecosystem and its biodiversity.

Recent estimates in Ganga Basin reveal a concerning 24.37 % contraction in GD range [6] and a population decline of over 50 % since 1957 [2,7]. Despite stringent regulatory conservation measures implemented by the Indian government, the species remains highly susceptible to anthropogenic threats, including bycatch mortality, poaching, boat traffic, a compromised (both in quantity and quality) prey base, climate change, flow modification by dams and barrages, and continual exposure to diverse pollutants [2].

Riverine cetaceans, including the GD, are exposed to various chemical pollutants primarily via dietary intake or offloading to the next generations via gestation and lactation [8]. With their extended lifespans, status as apex predators within local food chains, and high lipid reserves, GD often encounter significant health risks due to the substantial accumulation of various contaminants [8–10]. While studies on habitat modification, direct mortality, habitat loss, and overexploitation are frequently addressed [5,6,11–13], there remains a significant gap in understanding how chemical pollutants contribute to the decline of GD population, potentially undermining conservation efforts.

Within the broad spectrum of pollutants, Endocrine-Disrupting Chemicals (EDCs) have emerged as a critical ecological concern due to their persistent, bioaccumulative, mobile, and toxic properties. EDCs comprise a highly diverse class of compounds, including natural hormones and man-made substances such as pesticides, pharmaceuticals, plasticizers, potentially toxic elements, industrial chemicals, surfactants, and household and personal care products [14].

Over the past three decades, our understanding of the hazards and ecological risks associated with EDCs has advanced considerably, notably their correlation with disruptions in reproduction and development, that may result in observable regional population shifts in some aquatic mammalian species [15–18].

Despite global evidence on EDCs impact on aquatic mammals' health, there has been no assessment of their risk to GD, potentially compromising the efficacy of ongoing conservation efforts. While research exists on GD's exposure to some EDCs, they only cover the period from 1988 to 1996 [9,10,19–25]. This gap indicates that recent changes in EDC levels over the past few decades remain undocumented and unanalysed, resulting in significant gaps in our understanding of current risks and potentially leading to inadequately informed and insufficient conservation efforts. To develop targeted conservation interventions that protect this endangered species

Table 1

Details of fish species collected, across the three sites, with average length, average weight, trophic level and habitat preference.

Species	No.of Individuals	Niche	Feeding habit	Trophic Level	Average length (cm)	Average weight (grams)
<i>O. niloticus</i>	6	Benthopelagic	OV	2	22.55 ± 2.55	226.51 ± 77.58
<i>R. corsula</i>	11	Pelagic	OV	2.4 ± 0.2	21.5 ± 4.27	32.27 ± 5.18
<i>S. aor</i>	5	Demersal	CV	3.6 ± 0.53	29.25 ± 1.50	145.00 ± 11.37
<i>N. caelata</i>	3	Demersal	CV	4 ± 0.64	23.5 ± 3.56	152.41 ± 8.23
<i>C. latius</i>	8	Benthopelagic	HV	2.3 ± 0.2	13.45 ± 2.69	26.67 ± 3.82
<i>A. morar</i>	45	Benthopelagic	OV	3.2 ± 0.4	10.17 ± 1.41	8.78 ± 1.46
<i>R. rita</i>	12	Demersal	CV	3.7 ± 0.57	19.8 ± 3.82	77.54 ± 34.65
<i>L. rohita</i>	5	Benthopelagic	OV	2.2 ± 0.12	27.7 ± 1.44	301.11 ± 23.48
<i>M. armatus</i>	7	Demersal	CV	2.8 ± 0.27	13.2 ± 2.58	9.08 ± 1.35
<i>J. coitor</i>	6	Demersal	CV	3.4 ± 0.5	16.22 ± 1.25	31.88 ± 6.22
<i>S. bacaila</i>	10	Benthopelagic	CV	3.2 ± 0.4	12.87 ± 1.01	10.77 ± 2.41
<i>L. calbasu</i>	5	Demersal	HV	2	29.75 ± 1.52	311.33 ± 36.51
<i>M. cavasius</i>	14	Demersal	CV	3.4 ± 0.4	12.17 ± 2.76	8.33 ± 2.68
<i>C. reba</i>	7	Benthopelagic	OV	2.5 ± 0.2	19.39 ± 2.45	53.36 ± 29.92
<i>C. mrigala</i>	5	Demersal	OV	2.3 ± 0.2	28.45 ± 1.24	271.44 ± 8.38
<i>C. catla</i>	5	Benthopelagic	OV	2.8 ± 0.22	22 ± 2.83	147.65 ± 41.37
<i>L. bata</i>	6	Benthopelagic	HV	2	28.58 ± 1.73	230 ± 22.08
<i>C. chagunio</i>	4	Demersal	OV	2.8 ± 0.3	19.2 ± 1.63	60 ± 4.24
<i>L. pangusia</i>	6	Benthopelagic	HV	2	26.17 ± 0.91	166.67 ± 16.44
<i>C. garua</i>	13	Demersal	CV	3.73 ± 0.59	19.1 ± 4.01	43.67 ± 17.93
<i>B. dario</i>	4	Demersal	OV	3.2 ± 0.4	7.5 ± 0.28	8.36 ± 0.36

*OV=Omnivore; HV=Herbivore; CV=Carnivore.

Trophic level information is retrieved from FishBase [38].

and maintain ecological balance in their riverine habitats, it is crucial to urgently understand the health risks posed by EDC exposures to GD and its populations.

Biomonitoring of EDCs in GD can reveal health risks, but regulatory, ethical, and technical challenges complicate this traditional method. In the past two decades, the screening-level ecological risk evaluation (SLERA) approach based on dietary exposures has been successfully used as an indirect and non-invasive alternative to explore the potential health risk posed by contaminants to threatened aquatic mammals [26–28].

Given the predominantly piscivorous nature of GD, assessing EDC levels in fish facilitates the evaluation of potential health risks associated with EDC exposure in this species. Furthermore, studies suggest variations in EDC accumulation in fish is driven by biological traits, niche, feeding preferences, and geographical influences, these patterns seldom adhere to consistent trends [29–31]. Assessing these factors is vital for understanding biomagnification within the food web; however, limited efforts have been directed toward investigating these dynamics in GD habitats.

Given the above, the study aims to address the following key research questions: (1) what is the current extent of EDC contamination in the prey base of GD? (2) what factors contribute to the observed bioaccumulation patterns? and (3) what risk do EDCs pose to GD through dietary pathways? The overarching objective of this study is to screen for EDCs that pose risk to GD. Additionally, the study proposes an issue-based framework for monitoring and assessment of chemical pollution in GD and their habitats.

2. Study area

The investigated study area is a 60 Km stretch of Middle Ganga Reach (MGR) that lies between north latitudes 26° 33' 40.608" N–25° 34' 56.64" N, and east longitudes, 80° 18' 3.096" E – 83° 36' 6.48" E in the Varanasi district of Uttar Pradesh, India (Fig. 1). The stretch holds immense religious, economic, and socio-cultural importance and is an important habitat for diverse flora and fauna including GD [2]. Within the Varanasi district, there are 38 towns and 1327 villages, with a population of 3,676,841 (urban: 43.44 % and rural: 56.56 %) projected to reach approximately 4,300,000 in 2023 [32].

Varanasi, one of the oldest continuously inhabited cities globally, confronts obstacles in modernizing its sewage infrastructure owing to spatial limitations and preservation issues tied to its historic design. The river's catchment area, characterized by rapid development and high population density, coupled with poor wastewater management, frequently results in the discharge of untreated wastewater through multiple point-sources [33]. Meanwhile, its vast agricultural landscape, including cultivation on dry riverbeds, contributes as non-point sources of EDCs such as organochlorine pesticides [34,35]. The primary industrial sector is textiles, which holds a prominent position in the local economy, after tourism. Tourism also plays a central role in Varanasi's economy, with an annual footfall exceeding 9 million from domestic travelers and 1 million from international visitors [35]. The study area features three major sewage discharge points: Nagwa Drain (formerly Assi River), the Khirkia Drain, and the Varuna River (Fig. 1). Both Varuna and Assi rivers, often referred to as drains due to their poor water quality, receive significant wastewater and sewage inflow from numerous industrial and municipal drains, ultimately discharging into the Ganga River.

2.1. Sample collection

To assess the impact of urban settlements on the health of GD within conservation priority habitats, three sampling sites (S1, S2, and S3) were selected for EDC monitoring in the MGR. S1 and S3 predominantly represent agricultural landscapes and rural settlements, while S2 is characterized by urban and industrial settlements (Fig. 1, Fig. S1 and Table S1).

The *Platanista* genus exhibits a preference for prey based on size rather than species, primarily due to their narrow oesophagi, and species richness [36]. Typically, their prey size distribution is dominated by items measuring 20–30 cm [12,37]. Consequently, our sampling efforts were focused on collecting specimens falling within these preferred size ranges. Sampling was carried out in March 2021 and a total of 187 prey fishes of 21 species were obtained. Prey fish samples were obtained onsite from local fishermen as part of their routine catch for commercial purpose. Review and/or approval by Institutional Animal Ethical Committee (IAEC), Wildlife Institute of India was not needed for the study design.

The species were identified and their length and weight were recorded on-site.

The samples were packed in pre-cleaned aluminum sealed bags and kept in an ice box for transportation to the laboratory, where they were stored in a deep freezer at -20°C until further processing. Individual whole fish of the same species from each site were pooled and homogenized in a customized stainless steel tissue homogenizer. Details of the fish species collected from each site are given in Table 1. Data on trophic levels and habitat preference of each species were obtained from the FishBase [38].

3. Materials, extraction, and analyses

3.1. Chemicals and reagents

EDCs were selected mainly based on their major categories, and potential toxic impacts on aquatic mammals. Thirty-nine EDCs, including seven plastics additives - six phthalate (PAEs, the sum expressed as ΣPAEs): Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Di-n-butyl phthalate (DnBP), Butyl benzyl phthalate (BBP), Bis(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DnOP) and Bisphenol A; Seven organochlorine pesticides: p,p'-DDT(1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene), p,p'-DDE (1,1-Dichloro-2,2-bis (4-chlorophenyl) ethene dichloro diphenyl dichloroethylene), p,p'-DDD (1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) -the sum expressed as ΣDDTs ; four Hexachlorocyclohexane (HCHs; sum expressed as ΣHCHs) isomers: α -HCH, β -HCH, γ -HCH, δ -HCH; plasticizer: ; two household and personal care products (the sum expressed as ΣHPCPs): Triclosan (TCS), Triclocarban (TCC); Nineteen polychlorinated biphenyls (the sum expressed as ΣPCBs): PCB 8, PCB 28, PCB 44, PCB 52, PCB 77, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 126, PCB 138, PCB 153, PCB 156, PCB 167, PCB 169, PCB 180, PCB 189, PCB 209, and four potentially toxic elements (PTEs): Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As) were selected for present study.

Biota samples were spiked with internal standards and allowed to equilibrate for 4 h at room temperature (25°C). Sample pretreatment was conducted according to previously documented methods for persistent organochlorines [39,40], BPA and HPCPs [41, 42], and PAEs [43], with minor modifications for enhanced recoveries. The extracts were identified and quantified by Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC–MS/MS), Gas Chromatography–Tandem Mass Spectrometry (GC–MS–MS), and Inductively Coupled Plasma Mass Spectrometry (ICP–MS). Relative recoveries for OCPs and PCBs ranged 81–108 % and 91–103 % respectively. The average recoveries for PAEs, BPA, and HPCPs were 80–110 %, 78–112 %, and 81–108 % respectively. The percentage recoveries of the PTEs in the SRM ranged from 90.9% to 106 %. Detailed pretreatment methods, clean-ups, instrument parameters, quality assurance and quality control are detailed in Text S1 and Table S2.

3.2. Risk assessment for GD

Two dietary tissue guidelines—the reference dose (RfD; $\text{mg kg}^{-1} \text{ww day}^{-1}$) and the toxicity reference value (TRV, $\text{mg kg}^{-1} \text{ww day}^{-1}$), previously used for humpback dolphins [44,45]—were adapted and applied to assess potential health hazards to GD from consuming EDC-contaminated prey.

The TRV for EDC in GD was calculated based on the no observable adverse effect dose (NOAEL_r) for mammalian test species, and body weight scaling procedure (bodyweight of the dolphins/bodyweight of the test species) [44,45].

For dose-response assessment, the methodology detailed elsewhere [44,45] is utilized to derive a maximum allowable concentration (MAC) based on the Reference Dose (MAC_{RfD}) and Toxicity Reference Value (MAC_{TRV}) for a specific EDC in prey fish tissue. The MAC_{RfD} and MAC_{TRV} represents the highest concentration of each toxicant that can occur in the prey without causing harm to the species, and are derived using variables dependent on biological parameters of GD. The calculated values of MAC_{RfD} and MAC_{TRV} for GD is provided in Table S3.

The risk quotient (RQ) was determined from the ratio of the observed concentration and the MAC of a specific EDC in fish for GD consumption. The values of $\text{RQ} > 1$, $0.1 < \text{RQ} < 1$, and $\text{RQ} < 0.01$ indicate high, medium, and low dietary exposure risks of EDCs to the GDs, respectively [46]. The limited availability of comprehensive biological data on these dolphins may restrict the accuracy of SLERA. This limitation arises from uncertainties in exposure scenarios, including reliance on standardized parameters such as consumption habits, body weight, exposure frequency, fraction ingested, and exposure duration. Despite these limitations, the SLERA methodology adopted in this study aims to approximate a worst-case scenario, opting for a more conservative approach to ensure a higher level of protection for the threatened dolphins.

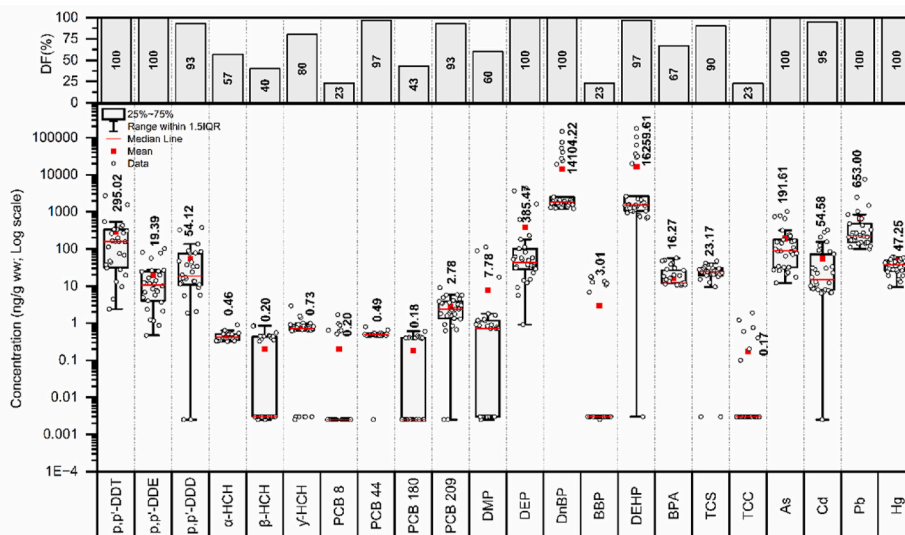


Fig. 2. Average concentration (ng/g ww; log scale) and detection frequency (DF%) of EDCs in prey of GD.

3.3. Statistical

Chemical concentrations were expressed as the range; average \pm standard deviation, and reported as ng/g wet weight (ng/g ww). To prepare data for analysis, data below the LOQ were adjusted to $\frac{1}{2}$ LOQ [47,48]. Assumptions were tested with Levene's tests for homogeneity of variance and Shapiro–Wilk tests for normal distribution. Non-parametric equivalents were used as the assumptions were violated. Kruskal-Wallis H-test was conducted to test variations of statistical significance for bioaccumulation patterns among sites, niches, and feeding behavior. Spearman's rank coefficient was conducted to evaluate the role of ecological factors (Trophic level) and biological traits (average weight and length) in bioaccumulation of EDCs. Statistical tests results were considered significant at p -value < 0.05 , and < 0.01 . Values were log-transformed for linear model application to assess relationships between tissue contaminant concentrations and species trophic levels.

4. Results and discussion

4.1. Bioaccumulation of multi-class EDCs in prey of GD

The average concentration and prevalence (detection frequency, DF) of multi-class EDCs in prey of GD is presented in Fig. 2. The dominant EDCs were DEHP (681.23–174990 ng/g ww) and DnBP (1218.07–146814.29 ng/g ww) accounting for 52.2 % and 45.3 % of total EDC burden, respectively.

The concentrations of DEHP and DnBP were followed by Pb, DEP, DDTs, As, Cd, Hg, TCS, BPA, DMP, PCBs (PCB 8, 44, 180, 290), BBP, HCHs, and TCC whereas DnOP and other target PCBs were below the method detection limits ($< DL$) in all the samples. Among the investigated freshwater fish species, *C. latius* (As), *A. morar* (Cd, Pb), *L. rohita* (TCS), *C. garua* (Hg), *R. corsula* (DDTs, PCBs, HCHs, DEHP), *R. rita* (BPA), and *S. aor* (DnBP) *S. bacaila* (TCC), and *C. reba* (BBP) exhibited the highest contamination for various categories of EDCs.

While high EDC concentrations were generally observed in prey fish from S3, likely owing to the downstream influence of both the point-sources at S2 and non-point sources at S3 itself, a Kruskal-Wallis test revealed no significant ($p > 0.05$) variations across the three locations.

The subsequent section provides group-wise bioaccumulation profiles of EDCs in prey of GD.

4.1.1. Persistent organochlorines

Bioaccumulation potential of three persistent organochlorines-DDT, HCH, and PCB on the GD prey were explored in this study (Fig. 2), with DDT (98.7 %) notably showing significant ($p < 0.05$) predominance, then Σ PCBs (0.97 %) and Σ HCHs (0.32 %).

The observed variances in the bioaccumulation patterns are consistent with a previous study on GD and its prey in the Ganga River [9], where DDTs were noted as the highest residue in GDs (~ 1300 ng/g wet weight), significantly surpassing PCBs and HCHs by a factor of 16.

The Σ DDTs concentration in the fish, collected from all three locations ranged from 2.87 to 3129.80 ng/g (368.53 ± 634.67 ng/g ww), with p,p' DDT ($p < 0.001$) as the major contributor (76 %) towards Σ DDT load, followed by p,p' DDD (16 %) and p,p' DDE (8 %). Kannan et al. [9] found significant DDT concentrations in the prey fish from the gut of GD, indicating considerable exposure to DDT through their diet. Notably, our study reveals that DDT concentrations in GD prey are twice as high as those reported by Kannan et al. [9]. This suggests a potentially high accumulation in GD, particularly given their apex predator status and limited capacity to

metabolize DDTs.

The presence of DDD as second most abundant metabolite indicates anaerobic degradation or dechlorination of DDT in the environment [49]. In the present study, the DDE/DDT ratio were consistently low (0.03–0.4) in prey fish suggesting recent DDT inputs [50]. The use of DDT in agriculture has been banned in India since 2006, with its application restricted to public health programs aimed at controlling vectors of malaria, dengue, kala-azar, and similar diseases [51].

Nevertheless, despite restrictions, fresh DDT inputs persistently appear in various aquatic compartments of Ganga River, prompting apprehensions about their illicit use in the catchment [52]. Further, the application of DDT in indoor residual spraying for the control of disease vectors seems to represent another potential source of DDT inputs into freshwater environments [52]. An increased surveillance and regulatory measures are imperative to guarantee the prudent and effective utilization of DDT in disease vector control programs.

The Σ PCBs concentrations in the all the fish samples ranged from <DL–10.47 ng/g ww (3.65 ± 2.20 ng/g ww). Notably, Σ PCBs were detected in prey fish species with concentrations almost 100 orders of magnitude lower ($p < 0.001$) than Σ DDT, and twice as high ($p < 0.001$) as Σ HCHs. In India, PCBs are highly regulated under the Environment Protection Rules [33], which prohibit the manufacture, import, export, and use of equipment containing PCBs [33].

The dominant PCB was PCB-209 (69 %) followed by PCB-44 (20 %), PCB-8 (6 %), and PCB 180 (6 %), while all other investigated PCBs were <DL. The potential sources of PCB-209 and other have been reported with the inadvertent or unintentional formation in specific organic pigment categories, including phthalocyanine green, dioxazine, azo, isoindolinone (PCB 8), monoazo (PCB 8), Titanium dioxide (PCB 209, and 180) and polycyclic pigments [53] [54,55]. The study area, particularly Site 2, is a prominent textile hub in the country, appears to be the primary source of unintentionally produced PCBs (UP-PCBs) attributed to the utilization of pigments in its manufacturing processes. Other potential inadvertent sources for the detected UP-PCBs include paint used on boats, printed material, and newsprint [56]. The presence of PCB 44, which undergoes dechlorination in anaerobic conditions [57], might be further explained by the high effluent load entering through three drains in S2. A similar pattern for PCB 44 has also been identified in other effluent-impacted Indian rivers [58].

Based on the findings of this study and the low metabolic capacity of GDs towards PCBs [9], it is recommended to consider UP-PCBs (including PCB-11, 52), in routine monitoring programs. Additionally, further confirmation studies in the fields of toxicology, environmental monitoring, and chemistry are needed to comprehensively understand the dynamics and impacts of UP-PCBs in GD and its habitats.

The Σ HCH concentrations, in prey fish ranged from <DL to 4.689 ng/g ww (1.22 ± 0.94 ng/g ww). The γ -HCH (50 %) concentration was major contributor towards Σ HCH, followed by α -HCH (25 %) and β -HCH (25 %), while δ -HCH levels in all fish species were <DL (Fig. 2).

Compared to DDTs and PCBs, HCH concentrations were notably low, a trend attributed to their low usage, relatively high volatility, lower bioconcentration factors, limited bioaccumulative capacity, and relatively biodegradable nature in the aquatic food chain [9, 50]. India banned technical HCH in 1997 (a mixture of α -HCH = ~70 %, β -HCH = 5–12 %, γ -HCH = 10–15 %, and δ -HCH = 6–10 %), whereas lindane (~99 % γ -HCH) was banned for manufacturing, import, or formulation in 2011 and for use in 2013 [51].

Furthermore, the α -HCH/ γ -HCH ratio, with values typically ranging from 3 to 7 in technical grade HCH, is used to assess the nature of HCH contamination with ratios below 3 indicating recent inputs of Lindane or γ -HCH. Interestingly, compared to previous studies [9,10], the HCH isomeric patterns in the present study were observed to be that of lindane (α -HCH/ γ -HCH < 3) rather than the technical HCH formulation. This finding aligns with our previous study conducted on the surface waters of Ganga River [52], where high concentrations of γ -HCH were observed. It suggests an association with the illicit use of this chemical in paddy and wheat fields during the flowering season, as well as in dry riverbed cultivation practices [52].

Despite lower concentrations of PCBs and HCHs compared to DDTs, the combined effects of these persistent organochlorine mixtures on exposed biota can be more complex than expected, potentially leading to compromised health and population-level consequences [59,60]. Additionally, the high transfer rate (~60 %) of organochlorine residues from mother to offspring, a characteristic unique to cetaceans, presents a considerable risk, especially to calves [8,21]. Notwithstanding regulatory measures and improvements in freshwater conditions, the persistence of these organochlorines could lead to enduring multi-generational toxic effects on dolphin populations.

4.1.2. Plastic additives

The cumulative concentration of 6 PAEs (Σ PAEs) in prey of GD ranged from 1581.21 to 254266.28 ng/g ww (30760.09 ± 65887.92 ng/g ww). DEHP and DnBP were the predominant (DEHP-53 %; DnBP-46 %) and prevalent (DF(DEHP)-100 %; DF(DnBP)-100 %) phthalates in all the fish samples, driven by their widespread production and usage, with environmental dispersion occurring at every stage of their life cycle [61]. BPA concentrations in prey fishes varied from <DL to 56.66 ng/g ww, (16.26 ± 16.75 ng/g ww; DF:67 %). Compared to phthalates, BPA is less prevalent in the environment likely due to its chemical embedment within products, which poses challenges for direct volatilization and leaching [62] Fig. 2.

Our results are consistent with recent findings by Chakraborty et al. [61], which reported dominance of DEHP and DnBP in the surface waters of the MGR and high concentration of BPA (4.46 μ g/L) in Varanasi.

The densely populated catchment area of the MGR suffers from inadequate solid waste management, which can lead to leaching of plastics additives due to extensive dumping of plastic water bottles, single-use polyethylene bags, and food packaging materials [35,61, 63]. Additionally, the high tourist influx may inadvertently contribute to littering, either from unawareness or a lack of proper disposal options, further straining the local waste management infrastructure, particularly during peak seasons. The textile industries in the catchment also appear to be a potential source of phthalates. These industries use various classes of chemicals, including phthalates,

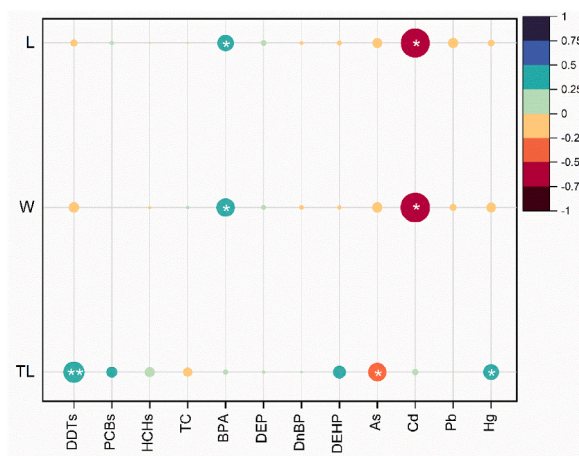


Fig. 3. Correlation of tissue EDCs concentrations with ecological factor (TL:Trophic level) and biological traits (W: Average weight; L: Average Length) Correlation significant at * = $p < 0.05$; ** = $p < 0.01$.

throughout their manufacturing processes, potentially leading to the discharge of these chemicals into the MGR [35].

4.1.3. Household and personal care products (HPCPs)

Commonly found in a variety of HPCPs, including hand soaps, toothpaste, detergents, plastics, and cosmetics, TCC and TCS are two polychlorinated aromatic antimicrobials that have been in use for decades. The escalating demand and production of these chemicals unavoidably lead to their ubiquitous presence in various environmental compartments [64–66].

The \sum HPCPs concentration detected in fish samples ranged from <DL to 47.05 ng/g ww (23.17 ± 10.99 ng/g ww). Interestingly, TCS was recorded to be the predominant (99.26 %) and prevalent (90 %) HPCP in all the fish samples compared to TCC (DF:23 %) as shown in Fig. 2. The variation in concentrations between the two antimicrobial agents could be linked to their distinct consumption patterns, chemical characteristics, and degradation rates in environmental matrices.

Triclosan is commonly found in liquid soaps, whereas triclocarban is mainly used in solid soap bars, which could account for their varying presence. Additionally, our sampling time (March 2021) coincided with the peak of the COVID-19 pandemic when enhanced hygiene practices spurred increased demand for liquid soaps, typically high in triclosan [67]. This surge likely led to significant triclosan discharge into aquatic systems, contributing to the observed bioaccumulation and variations.

4.1.4. Potentially toxic elements (PTEs)

PTEs such as Pb, Hg, Cd, and As are recognized as EDC in both humans [68–70], and aquatic mammals [71–74].

In the present study, Pb was the most dominant PTE with concentrations ranging from 100 to 7550 ng/g ww. In order of decreasing rank, concentrations of Pb were followed by As (12–1007 ng/g ww), Cd (0.25–322 ng/g ww), and Hg (9.4–374 ng/g ww) as presented in Fig. 2.

Similar bioaccumulation patterns of these PTEs have also been noted in the tissue of the GD [19].

The accumulation of Cd, and Pb in fish samples points towards their industrial origins, including industries like textile, dyeing, electroplating, metallurgy, etc. Additionally, the presence of busy national highways and heavy boat traffic at all monitoring stations, along with long-range atmospheric transport of Pb, suggests contributions of Pb emissions from both land-based and river activities [75].

PTEs such as Cd, and Pb are also present in small quantities within chemical additives used in plastics, to enhance the functional and aesthetic attributes of these materials [76]. The incorporation of PTEs in these additives, coupled with the improper utilization, recycling, and disposal of plastics, raises concerns about the potential unintentional release of PTEs into freshwater environments [76]. The accumulation of arsenic (As) in fish species may be associated with the application of phosphate fertilizers containing high levels of As [77]. These fertilizers, characterized by elevated arsenic concentrations, could potentially enter the river during flood events.

4.2. Relationships of EDCs concentrations with ecological, and biological factors

A Spearman's correlation analysis indicates a significant direct relationship between DDTs ($p < 0.01$) and Hg ($p < 0.05$) accumulation with trophic level (Fig. 3). In contrast, As ($p < 0.05$) shows an inverse relationship with the trophic level of the species. These results are further supported by linear modelling (Fig. S4) that revealed significant effect of trophic levels on the accumulation of DDTs ($R^2 = 0.24$), As ($R^2 = 0.22$) and Hg ($R^2 = 0.14$) in fish tissues, indicating biomagnification for DDTs and Hg, but trophic dilution for As. Biomagnification of DDT and mercury in the riverine food web of the present study, is consistent with observations by other authors [78,79]. As has been reported to dilute through the food chain, owing to the ease of oxidation of As(III) to As(V) compared to the methylation of accumulated As in organisms with increased trophic levels [80]. No significant relationships were observed for PCBs,

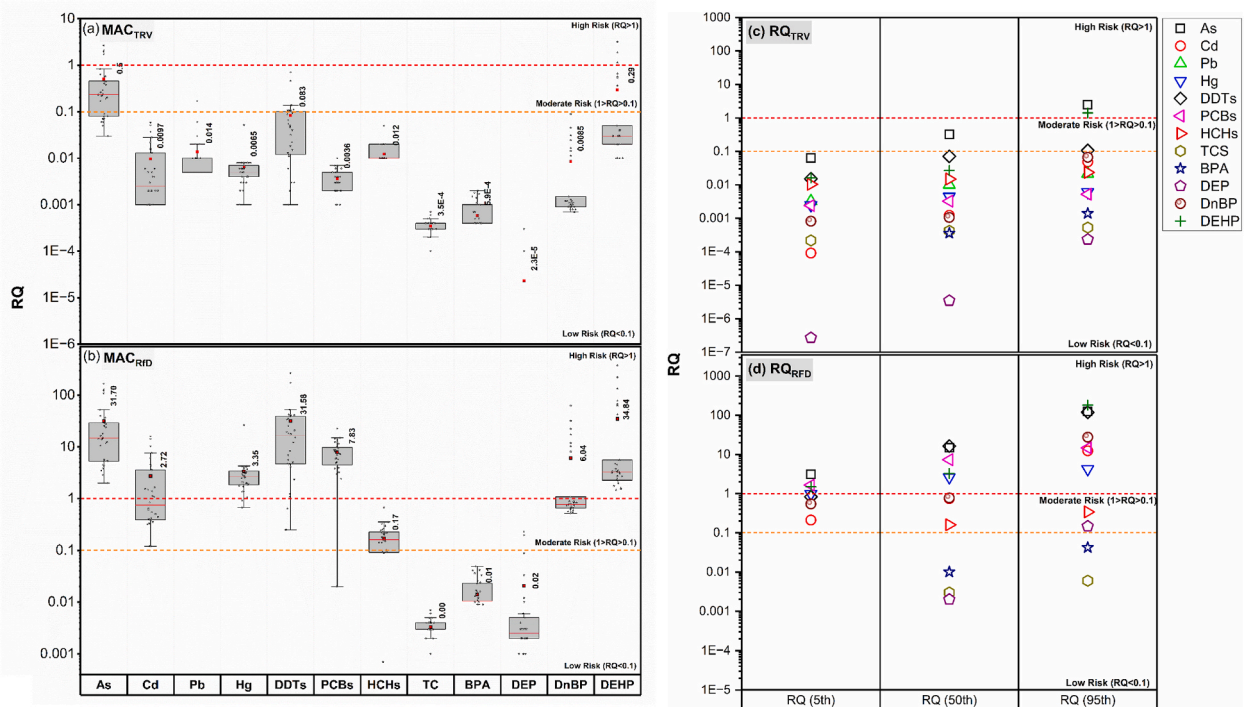


Fig. 4. Risk assessment (RQ-log scale) of EDCs to GD MAC_{TRV} and MAC_{RFD} based on average concentrations (a–b) and 5th, 50th, and 95th percentile data (c–d).

DEP, Cd, and, Pb, whereas non-linear relationship between trophic levels of the species and HCHs, BPA, DnBP and DEHP were noted. These findings suggests that contaminant dynamics may not always be simple enough to be explained by linear regressions and simplification by process selection, and assumptions of equilibrium in predictive models can considerably affect the reliability and predictive power of models [81]. Additionally, as bioaccumulation and biodilution depend on various factors including local food webs and environmental factors, thus further investigations are necessary for effective modeling, prediction, and risk assessment.

A Spearman's correlation (Fig. 3) analysis indicates a significant correlation of biological factors +with BPA and Cd. BPA accumulation shows positive relationship with average length and weight, consistent with the results Zhou et al. [82]. Studies on other phenolic compounds show decreasing toxicity with increasing fish size [83], however, knowledge gaps pertaining to the relationship of BPA accumulation and size of the species, need to be addressed to understand its risk to piscivores with specific prey size preferences. An inverse relationship of Cd with both length and weight of the species is observed. Similar relationships have been observed in previous studies, and especially in smaller individuals [84]. The higher significance of smaller individuals for the accumulation and toxicity of these compounds could potentially increase the contamination risk to GD, whose prey preference is limited by size.

Nevertheless, it is important to consider that both length and weight may serve as proxies for various factors such as species, sexual maturity, health, diet, and habitat quality, thus a thorough investigation is warranted to understand such dynamics [85,86].

4.3. Relationships of EDCs concentrations with niche and feeding behaviour

A significant difference ($p < 0.05$) in bioaccumulation of DEP and DEHP was observed between pelagic and benthopelagic fish (Fig. S2). A significant variation was also observed in As accumulation between pelagic and benthic, and benthopelagic and benthic species. For all three of these contaminants, pelagic fish species showed the highest bioaccumulation, indicating their uptake from organisms of the pelagic food web such as algae and zooplankton [87–89].

With regards to feeding behaviour, As was the only EDC that showed a significant ($p < 0.05$) variation in accumulation between herbivorous, carnivorous and omnivorous species (Fig. S3). Omnivorous species accumulated higher concentrations of As on average, whereas carnivores reported the lowest concentrations. As discussed in the previous section, this may be owed to the ease of oxidation compared to methylation of As in carnivores [80].

4.4. Risk assessment of EDCs exposure to GD

The current study relies on the dietary tissue residue guidelines method to assess the health risks that GD may encounter due to exposure to EDCs. As toxicological data were only available for a select group of EDCs, risk assessments were not carried out for those lacking such data.

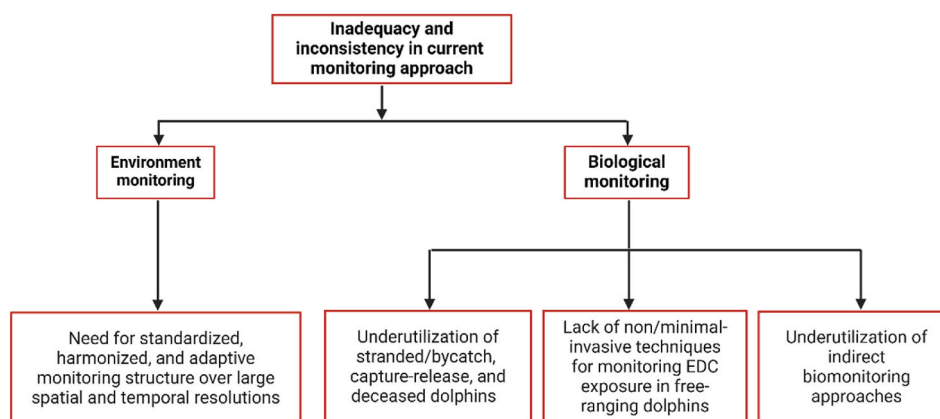


Fig. 5. Identified issues in the current approach for monitoring chemical contaminants in Gangetic Dolphin and its habitats Color should be used for figure in print. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

A summary of the calculated Risk Quotients (RQs), based on MAC_{RfD} and MAC_{TRV} , that selected categories of EDCs in prey-fish species may pose to GD, is provided in Fig. 4a–d.

In general, the average risks associated with EDCs, as assessed by MAC_{TRV} , was consistently less than 1, with all RQs falling within the range of <0.001 to 0.51. The average risk associated with dietary exposure to DEHP and As was noted to be moderate (Fig. 4a), with data from the 95th percentile indicating high risk for both EDCs (Fig. 4c).

The RfD, commonly utilized in human health risk assessment, offers a more robust and conservative evaluation of potential adverse health effects associated with exposure to environmental contaminants, incorporating higher safety factors for added protection to threatened species.

Based on the MAC_{RfD} , DEHP > DDT > DnBP > As > PCBs > Hg > Cd, demonstrated a high average potential risk (RQ = 2.72–30.84) to GD through dietary intake whereas HCHs revealed moderate risk (RQ = 0.17). The substantially high RQs can be attributed to both the high toxicity of the chemicals and their widespread usage within densely populated, agriculturally dominated and industrially active catchment area. In the present study, the RQ values for TCS, BPA, and DEP remained below 1, indicating their low potential to pose a risk to GD.

Considering a worst-case scenario based on 95th percentile data, the RfD-based RQs for DEHP, DDT, and As exceeded 100, while ranging from 12.41 to 27.65 for DnBP, PCBs, and Cd.

To date, there are no known studies specifically investigating the dose-response relationships of contaminants on Gangetic dolphins. Hence, given the known health impacts of EDCs in other aquatic mammals including cetaceans, we can anticipate potential impacts on Gangetic dolphins.

The substantially high RQ_{RfD} for DEHP and DnBP may pose an elevated risk to these species, as phthalates and their metabolites can potentially affect thyroid function [90] and cause lipid disruption [91] in cetaceans.

Similarly, persistent organochlorines such as DDTs and PCBs have serious implications for cetacean health and populations around the world through endocrine disruption, carcinogenicity, cytotoxicity, reproductive impairments and immunosuppression [74,92–95]. PCBs and their alternatives have been recorded to significantly raise tetraiodothyronine, testosterone, and cortisol levels of the Indo-Pacific finless porpoise [74] and have been also associated with the population collapse of the common seal [16], and Killer whales (*Orcinus orca*) [96–98]. Additionally, the high tendency of these organochlorines for transplacental transfer raises further concerns about their potential to exert toxic effects on foetal growth and development [99].

PTEs such as Hg, Cd, As and Pb have been associated with various immunotoxic and neurotoxic effects in aquatic mammals [100, 101]. In St. Lawrence Beluga Whales, individuals with high Hg and Pb concentrations were also observed to have chronic lesions and reproductive impairment [102].

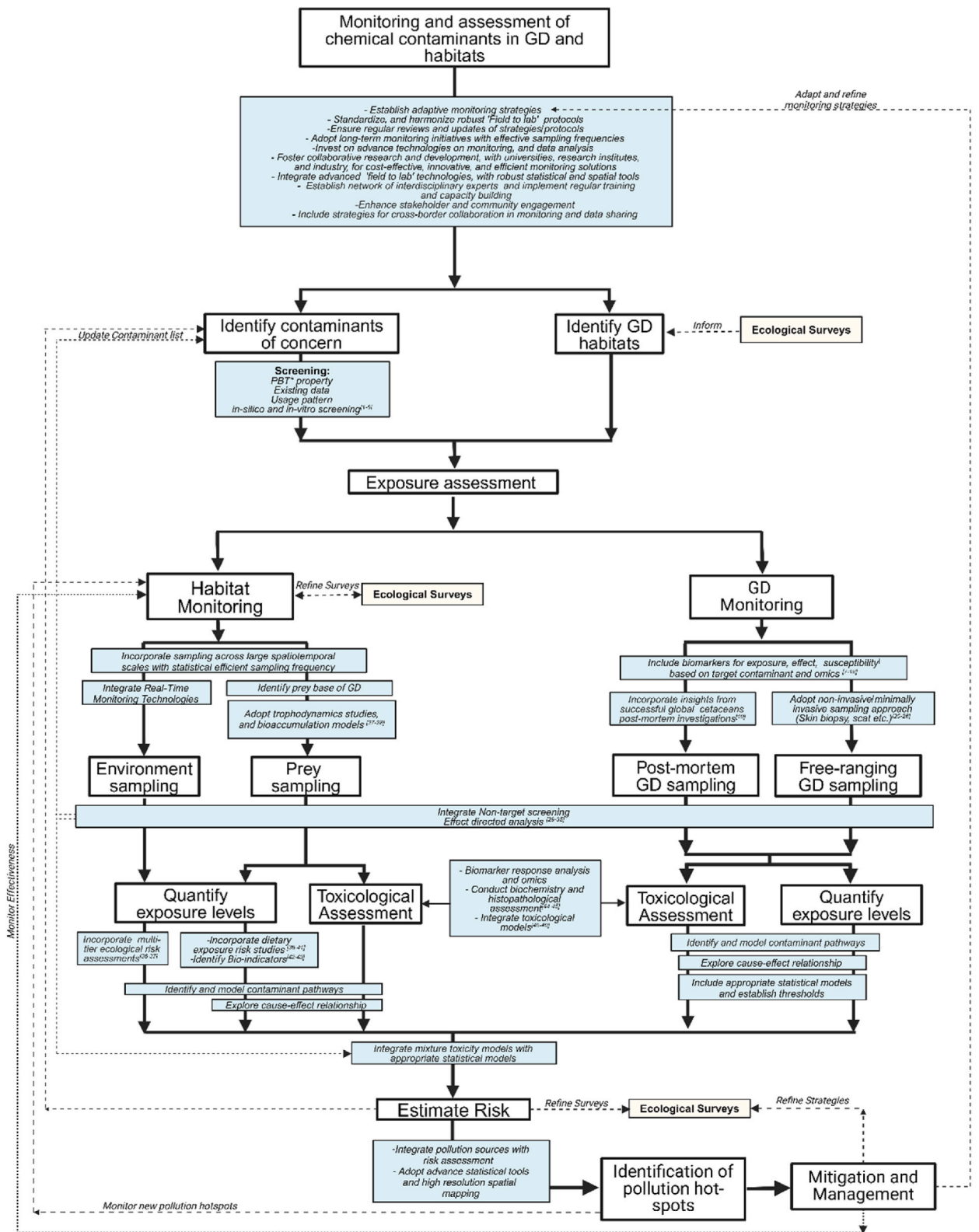
Although the current study indicates a low risk associated with TCS, TCC, and BPA, their known toxicity to coastal cetaceans [74, 103,104], along with their high production and widespread usage, justifies their inclusion in regular monitoring programs as a proactive measure.

Considering the significant risks that these EDCs pose to GD, as identified in this study, alongside their adverse effects on other aquatic mammals, it is imperative to deepen our understanding of these threats to GD for effective conservation.

A pivotal first step in addressing the threats from these EDCs could involve establishing a robust monitoring program to assess the levels and effects of these EDCs in GD and their habitat. Such a program would lay the groundwork for informed, effective conservation actions and policy development aimed at reducing pollution and protecting this umbrella species and its ecosystem.

4.5. Way Forward: Issue-based monitoring recommendations to strengthen existing GD conservation program

While marine cetaceans have greatly benefited from monitoring programs targeting chemical pollution threats [105–107], there is a marked lack of such monitoring program for GD highlighting a significant oversight in current conservation efforts. This gap in



(caption on next page)

Fig. 6. Proposed framework for monitoring chemical contaminants in Gangetic Dolphin and its habitats. Green box represents the pre-requisite deemed necessary for the successful implementation of the framework; yellow box represents contributions from ecological survey (such as habitats, population dynamics, distribution range, biology, and behaviours). Dashed lines represent secondary interactions between different components of framework. References 1–49 are provided in Supplementary Information. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

monitoring is particularly concerning given that a high extinction risk often coincides with low availability of data, especially those pertaining to dose and response [108]. Addressing this research gap is paramount for devising comprehensive conservation strategies that effectively mitigate chemical pollution threats to species, thereby enhancing their chances for survival and stability.

To address this, we identified the issues with the current approach for monitoring chemical contaminants in GD and its habitats (Fig. 5). Based on the identified issues, we propose an issue-based framework for monitoring and assessing chemical stressors affecting Gangetic dolphins and their habitats (Fig. 6). This framework is designed to provide relevant data on exposure and risk assessments, which are imperative for formulating informed conservation strategies and policy interventions.

Expanding on our earlier GD conservation guidelines [13], the proposed framework is a detailed strategy devised in collaboration with interdisciplinary experts. This tailored approach focuses on meeting the specific monitoring requirements of GDs and their habitats in response to the challenges posed by chemical pollution.

Furthermore, the proposed framework's development is informed by valuable insights from successful monitoring programs, reports, and publications globally that focus on the threats posed by chemical pollution to aquatic mammals, particularly cetaceans ([107,109–117]).

At each stage of this framework, specific prerequisites are identified and deemed necessary for the successful implementation of the framework. It is noteworthy that the framework is designed in such a way that its scope extends beyond riverine cetacean species other than those covered in the present study.

5. Conclusion

This study presents the first investigation into the health risks posed to Gangetic dolphins by multi-class EDCs.

Plastic additives, DEHP and DnBP, were the dominant EDCs in the prey fishes of GD. The study highlights the prevalence of unintentionally produced PCBs in the dolphin prey. Fresh inputs of p,p' DDT, and γ -HCH in GD prey indicates the ineffectual compliance with regulatory standards and policies at the regional level.

Bioaccumulation of some EDCs in prey fish were found to be dependent on foraging behavior (As), niche (DEP, DEHP, As), trophic level (DDT, As, and Hg) and size (Cd and BPA). The trophic magnification, displayed by DDT and Hg, is particularly concerning due to its potential for severe impacts on apex predators like GD. Agricultural settlements, vehicular emissions, improper plastic disposal, textile industry, and high tourist influx are identified as the primary drivers of EDCs contamination in MGR. Implementation of stricter regulations on industrial discharges, improving wastewater treatment infrastructure, and promoting sustainable practices in agriculture and industry are essential to mitigate the sources of these contaminants and minimize their impact on GD and their habitats.

A screening-level ecological risk assessment, utilizing both TRV and RfD, reveal varying levels of risk to GD through dietary exposure. While TRV-based risk for most EDCs were low, RfD-based RQ showed high risk to GD from DEHP, DDTs, DnBP, As, PCBs, Hg, and Cd. Given their known adverse effects on other mammalian species, especially cetaceans, these threats should not be overlooked. While the current study suggests a low risk associated with BPA, and TCS, TCC, considering their high production, widespread usage, and known toxicities, it is recommended to include these EDCs in regular, comprehensive monitoring initiatives as a proactive measure.

Efforts should focus on understanding the mechanisms of exposure, bioaccumulation, and toxicological impacts of EDCs on GDs and their habitats. Holistic investigations of EDCs and other contaminants of concern, across large temporal and spatial scales in GD and its habitats are also recommended.

Furthermore, the framework proposed in this study has the potential to enhance GD conservation efforts.

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CRedit authorship contribution statement

Ruchika Sah: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Gautam Talukdar:** Supervision, Writing – review & editing. **Megha Khanduri:** Formal analysis. **Pooja Chaudhary:** Data curation. **Ruchi Badola:** Funding acquisition, Project administration, Resources, Supervision. **Syed Ainul Hussain:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e35130>.

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