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EDITED BY
Nsikak U. Benson,
Topfaith University, Nigeria

REVIEWED BY
Youji Wang,
Shanghai Ocean University, China
Vimalkumar Krishnamoorthi,
New York University, United States

*CORRESPONDENCE
Stephanie L. Renkers,
srenkers@sfu.ca

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Investigating the ecological and toxicological significance of Cyanox®53 recovered from intertidal sediments and varnish clam

Stephanie L. Renkers* and Leah I. Bendell

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

We examined the ecological and toxicological implications of the microplastic, Cyanox®53, found in sediments and varnish clams across seven beaches in Burrard Inlet, British Columbia (BC). Using the simulation models embedded within Estimation Programs Interface (EPI) Suite™, the potential persistence, bioaccumulation, and toxicity of Cyanox®53 was assessed to evaluate the risk to varnish clams foraging on sediment containing this contaminant. Moreover, we used a bioenergetic model, based on the blue-listed surf scoter species, to estimate the risk of daily ingestion of Cyanox®53 per body weight in overwintering seabirds. Our findings indicate that varnish clams collected from Burrard Inlet accumulate on average 0.46 particles of Cyanox®53/clam, and based on bioenergetic modeling, results in surf scoters potentially consuming 78 (for males) to 83 (for females) pieces of Cyanox®53 daily from foraged varnish clams. EPI Suite™ predicted Cyanox®53 to be persistent, however, unlikely to bioaccumulate as a "traditional" chemical. Furthermore, the estimation of potential acute and chronic toxicity of Cyanox®53 to aquatic organism surrogates, such as fish, *Daphnia magna*, and green algae, was inconclusive due to model variability and limitations within EPI Suite™. To fully understand the potential risks of Cyanox®53 further investigation is warranted.

KEYWORDS

plastic additive, microplastics, Cyanox®53, environmental fate, toxicity, EPI suite TM, scoter duck bioenergetic model, varnish clams

1 Introduction

Microplastics (MPs) are generally characterized as any plastic particle ranging from 1 μm to 5 mm in diameter, and can present in all manners of shapes, sizes, and colors in the aquatic environment (Thompson, 2015; Hermabessiere et al., 2017; Crawford and Quinn, 2019; Bendell et al., 2020; Bermúdez and Swarzenski, 2021). The origin in which MPs can arise from also varies, with some emerging from weathered macroplastics (>1 cm) that have undergone fragmentation (e.g., fibres from fishing nets, synthetic clothing, or tire remnants), and others coming from manufactured products (e.g., preproduction plastic resin pellets, industrial abrasives, or microbeads in personal care products) that have entered the waterways from plastic litter, treated wastewater release, or sewer overflow (Baztan et al., 2017; da Costa et al., 2017; Hermabessiere et al., 2017; Crawford and Quinn, 2019; Department of Fisheries and Oceans Canada, 2021). Regardless of the source, albeit

noteworthy, MPs are now acknowledged as one of the most pervasive and formidable threats ever to endanger the marine environment (Crawford and Quinn, 2019). Whether it is due to not possessing a feeding mechanism capable of discriminating between prey and anthropogenic material, mistaking the MP as planktonic prey, or from ingesting prey containing MPs (i.e., trophic transfer), many aquatic organisms inadvertently ingest MPs as food (Moore, 2008; Lusher, 2015; Gallo et al., 2018; Luan et al., 2019). In some species, like shore crabs (*Carcinus maenas*) and blue mussels (*Mytilus edulis* L.), MPs can also be drawn into the body via gill cavity inhalation (Browne et al., 2008; van Moos et al., 2012; Watts et al., 2014; Lusher, 2015; Miller et al., 2017; Gallo et al., 2018). MP uptake has been observed in over 300 marine species, including zooplankton, benthic invertebrates, fish, sea reptiles, seabirds, and marine mammals (Gallo et al., 2018; Luan et al., 2019; Savoca et al., 2021). Without enzymatic pathways specific to synthetic polymer break down, the assimilation of MPs can inflict a variety of physical effects (depending on the size and shape) in most aquatic organisms, including gut obstruction and ulcerative lesions; weight loss from decreased food and nutrient intake; reduce respiration rates from MP entrapment in the gills; interfere with other tissues or organs (i.e., lysosomal, circulatory, hemolymph) via translocation; impact development, metabolic parameters, or cellular function; impair mobility, brain function and reproductive output; or reduce life expectancy (Browne et al., 2008; Teuten et al., 2009; Andrady, 2011; Besseling et al., 2013; Lambert et al., 2013; Wright et al., 2013b; da Costa et al., 2017; Law, 2017; Nelms et al., 2019; Hale et al., 2020).

MPs also pose the additional risk of exposing aquatic organisms to toxic chemicals associated with the plastic (Koelmans et al., 2014; Espinosa et al., 2016; Adam et al., 2019). Indeed, while plastic polymers are typically considered biochemically inert due to their large molecular size, they often contain hazardous non-polymeric substances (i.e., unreacted residual monomers, oligomers, catalyst remnants, polymerization solvents, and various chemical additives) deliberately added during processing to enhance the properties of the plastic and prevent 'aging' (Lithner et al., 2011a; Olabisi and Adewale, 2016; Gallo et al., 2018). In most instances, these substances are of low molecular weight and are either weakly or not completely bound to the polymer matrix (Organisation for Economic Co-operation and Development OECD Website, 2004; Lithner et al., 2011b). This increases their likelihood of leaching out of the plastic and into the surrounding medium until the appropriate octanol/water partition coefficient (K_{ow}) is reached (Andrady, 2011). Moreover, it is rare and quite unlikely for organisms to be exposed to only one chemical in isolation, raising the prospect of co-contaminants interacting with one another and potentially inducing additive or synergistic effects (Rochman, 2015; Baztan et al., 2017). Persistent organic pollutants (POPs), such as alkylbenzenes, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls, as well as inorganic contaminants like silver, titanium dioxide nanoparticles, barium, sulphur, and zinc from the environment have been known to absorb or concentrate in MPs (Teuten et al., 2009; Andrady, 2011; Fries et al., 2013; Khan et al., 2015). Evidence of non-persistent pollutants, such as commercially important plasticizers (e.g., dibutyl phthalate), or trace monomers like bisphenol A (BPA) and alkylphenols, have also been found at greater concentrations in MPs than in seawater or sediments, and are known to readily leach out and disrupt endocrine functionality of exposed organisms (Carlisle et al., 2009; ECHA, 2014; ECCC, 2018; Gallo et al., 2018). Even some synthetic phenolic antioxidants and related transformation products, that are normally used to impede oxidative reactions and extend plastic shelf-life, can exert hepatic toxicity, endocrine disrupting effects, and carcinogenicity if they were to release from the polymer matrix and become bioavailable within an organism (Lambert et al., 2013; da Costa et al., 2017; Liu and Mabury, 2020). Because of this, 'plastic microbeads' and 'plastic manufactured items' have been added to the list of toxic substances under Schedule 1 of the Canadian Environmental Protection Act (CEPA), and the composition and properties of most plastic additives have been revealed to public consumers (Espinosa et al., 2016; ECCC, 2020; ECCC, 2021; Plastic Soup Foundation, 2021). However, this is not the case for all as gaps in knowledge still remain for some compounds. For case in a point, and the focus of this study, limited information exists on the additive, Cyanox®53.

Cyanox®53 is chemically characterized as a white powdered alkylated bisphenol derivative, more specifically a primary (phenolic) antioxidant, with a specific gravity density of 1.74 (van Alphen and van Turnhout, 1973; McEntee, 1990; AccuStandard, 2018; Wang et al., 2019; Wiley, 2021). The additive was originally developed by Cytec Industries Inc. for the intended use of enhancing the stability of polymers (i.e., PE, PP, PS, and ABS) by providing protection against thermo-oxidative degradation that would otherwise weaken structural integrity or cause discoloration during thermal processing (Bolgar et al., 2016; Bendell et al., 2020; Solvay, 2020). Although the thermal stabilizer has since been discontinued and is no longer available for purchase in the public domain, the integration of Cyanox®53 has been documented in different products including rubbers, PE wiring and cable resins, and even in the packaging of feminine hygiene products (e.g., wrapper in organic cotton tampons) (Flick, 2001; Bolgar et al., 2016; Greenpeace Research Laboratories, 2019). Beyond these facts, however, little is known about Cyanox®53 as experimental data and model-based predictions are lacking. In fact, while information on other Cyanox® variants (e.g., Cyanox®2246, Cyanox®425, and Cyanox®1790) has been disclosed in an assortment of documents, literature regarding the ecological and/or toxicological significance of Cyanox®53 remains scarce. This is likely because particles like Cyanox®53 tend to get associated with the plastic polymers during MP surveys, rather than be classified as a true plastic product. As a result, their occurrence in the environment more often would go unreported. In the few times Cyanox®53 has been recorded in the environment, the antioxidant has been found to be disassociated from its inert carrier and abnormally presented as a white fibrous particle. For example, following a MP composition survey at inner Kongsfjord, Svalbard, the Norwegian Geotechnical Institute in collaboration with the Geographical Survey of Norway recovered white fragments of Cyanox®53, at a frequency 27%–64% of total MPs, in beach surface sediments at two sampling sites (Knutsen et al., 2019). Bendell et al. (2020) found fibrous particles of Cyanox®53, in addition to a high volume of MP fragments and spheres, in the digestive tracts of 26 varnish clams (*Nuttallia obscurata*) during an MP biomonitoring survey in Burrard Inlet, British Columbia (BC). Similarly, an investigation conducted by Achiluzzi (2022) recovered Cyanox®53 particle concentrations in varnish clams from False

Creek, Vancouver, BC. Based on their findings, Bendell et al. (2020) and Achiluzzi (2022) proposed that Burrard Inlet, BC could be geographically an important source of Cyanox®53 from sediment to higher trophic levels. Still, further investigation into the ecological and toxicological implications of Cyanox®53 is needed.

At present, assessment of the toxicity of MPs poses quite a challenge to researchers as they not only act as a physical obstruction but also as a dynamic vector and reservoir of inherent additives and anthropogenic contaminants capable of inflicting physiological and toxicological damage to organisms. Traditionally, *in vivo* experiments were used by scientists as the fundamental risk assessment approach in identifying the toxicological mechanism and structure–activity relationship for specific chemicals (Hayes and Kruger, 2014). However, within recent decades the issue of whole-animal toxicity testing has become more pressing and contentious as concerns over the welfare and misuse of animals have been raised (Goldberg and Frazier, 1989; Hayes and Kruger, 2014). Even global regulations, including European Chemical Agency, REACH initiative, U.S. Toxic Substances Control Act, and the Canadian Environmental Protection Act, that once mandated *in vivo* toxicity testing, now encourage an increased reliance on *in silico* approaches to predict the intrinsic activity and potential absorption, distribution, metabolism and excretion characteristics of chemicals (Madden et al., 2020; Zhou et al., 2021). As such, less costly and non-invasive approaches, such as Quantitative Structure-Activity Relationship (QSAR) modeling, have been utilized more frequently over traditional *in vivo* experiments in the identification of physiochemical properties, and toxicological mechanism and structure–activity relationship for specific chemicals (Lapenna et al., 2010; Hayes and Kruger, 2014; OECD, 2022a; OECD, 2022b). The application of bioenergetic models have also provided a valuable tool for identifying factors influencing the feeding and growth of aquatic organisms, as well as for quantifying their consumption under varying environmental stressors and ecological conditions (Enders and Scruton, 2006). Here, we use a similar approach to gain some insight into the potential toxicity of Cyanox®53 and possible risk if it were to leach from its solid form.

In this present study, we examined the ecological and toxicological implications of Cyanox®53 concentrations found in sediments and varnish clams (*N. obscurata*) from Burrard Inlet, BC. Our objectives were as follows: i) detect the presence of Cyanox®53 within intertidal surface sediment and adult varnish clams across seven beaches in Burrard Inlet, B.C.; ii) employ attenuated total reflectance-Fourier transform infrared (ATR-FTIR), electrospray ionization-liquid chromatography/mass spectrometry (ESI-LC/MS), and proton nuclear magnetic resonance (^{1}H-NMR) to propose a chemical structure for Cyanox®53; and from this structure, iii) estimate its environmental fate and potential toxicity via Estimation Programs Interface (EPI) Suite™, and the risk of daily ingestion of Cyanox®53 per body weight in overwintering seabirds using a bioenergetic model, with the surf scoter (*M. perspicillata*) as a representative species. The varnish clam was selected as it has shown to be an opportunistic feeder and effective biomonitor of MP pollution within sediments (Bendell et al., 2020). It is also well-established in the southern coastal regions of BC and represents a key diet item of the blue-listed surf scoter species, therefore making it an ecologically relevant choice for risk analysis (Gillespie et al., 1999; Gillespie et al., 2001; Palm et al., 2012; Brietzke and Starzomski, 2013; Chan and Bendell, 2013; Bendell et al., 2020). We predicted that Cyanox®53 would be the most abundant near the First Narrows strait (mouth of outer and inner harbor) where the majority of the Port of Vancouver is centralized. Secondly, we hypothesized that akin to other bisphenol-related compounds, Cyanox®53 may possess some degree of toxicity towards aquatic organisms and/or seabirds. As attention is often solely directed towards more commonly known MP polymers (e.g., polyethylene, polypropylene, and polyvinyl chloride), we aimed to gain a better understanding of the potential risk of Cyanox®53 to varnish clams and surf scoters given their important roles within the marine ecosystem.

2 Materials and methods

2.1 Study sites

Seven coastal sites in Burrard Inlet, BC (49.3174° N, 123.1913° W) were selected for sampling. These included Spanish Banks Beach (SB), Jericho Beach (J), English Bay Beach (EB), Third Beach (TB), Ambleside Park Beach (AP), Cates Park Beach (CP), and Barnet Marine Park Beach (BMP) (Figure 1) (Google Earth Pro, 2024). Descriptions, impact levels, substrate types, and GPS coordinates of each site are provided in the Supplementary Material.

2.2 Sediment analysis

2.2.1 Collection and preparation

Sampling was conducted in late May to early June during a period of maximum low tides. Approximately 3.5 kg of surficial sediment was collected via 'grab sampling' technique at a depth of 10 cm from each site and placed into a labelled plastic bucket and sealed for prevention of contamination. Following collection, sediment samples were transported back to the laboratory and sieved separately for particles <5 mm into a pre-weighed aluminum tray using CE Tyler Canadian Standard 4.75 mm sieve. Newly sieved sediment was then subdivided into three 1 kg samples per study site. Each subsample was homogenized before being placed into a Precision® Gravity Convection Incubator at 60°C for 48 h or until completely dried. Once dried, samples were set aside for tagging and separation.

2.2.2 Nile red tagging of particles

As described in Maes et al. (2017), a stock solution of zinc chloride ($ZnCl_2$) (granular ACS reagent with a purity of >97%) at a density of 1.38 g/mL was prepared gravimetrically at room temperature by combining 420 g $ZnCl_2$ with 700 mL analytical-grade water. Nile red dye (≥98.0% purity) stock solution in acetone (≥99.5% purity) was prepared to a final concentration of 10 µg/mL in a tinted vial and stored in a dark refrigerator at 4°C to prevent chemical degradation and microbial growth until tagging was conducted. Approximately 20 g of dry sediment was suspended in 30 mL $ZnCl_2$ solution and 2 mL Nile red solution within a 50 mL centrifuge tube. Samples were shaken for 30 min on a Precision®

FIGURE 1
Locations of the seven designated sampling sites in Burrard Inlet, BC. Yellow markers indicate sampling at AP, BMP, CP, EB, J, SB, and TB.

Scientific Dubnoff Metabolic Shaking Incubator at 100 rpm. These steps of solution prep and staining were repeated for all subsamples.

2.2.3 Particle density separation

Particles from sediment samples were separated by centrifuge based on the procedures laid out in Maes et al. (2017), with some minor modifications. Each centrifuge tube filled with the sediment mixture was centrifuged in a Beckman Allegra 64R centrifuge at 3900 *g* for 8 min with a braking speed of 9. The supernatant containing the stained particles was collected using a 5 mL glass Pasteur pipette and decanted through a Whatman™ 1001–090 Grade 1, Pore Size, 9 µm qualitative cellulose nitrate filter paper under vacuum filtration. This procedure of density suspension was repeated for each subsample. Dried filter papers were placed into Petri dishes and sealed with Parafilm® M all-purpose tape until inspection.

2.3 Bivalve analysis

2.3.1 Collection and preparation

Twenty-five adult varnish clams varying in size were collected at each site in late May to early June (Permit # XMCFR 16 2020) from a depth of approximately 10 cm in the mid to high intertidal zones (N = 175). All collected bivalves were secured with a thick rubber band around their shell to prevent the loss of any particle contents and then placed into their respective site labelled Ziploc® bag for ease of transportation. At the laboratory, biometric data of internal tissue weight, shell dimensions (length, height, width) and weight of each bivalve was measured, and a corresponding condition indices value was determined using Equation 1 (Benali et al., 2015). Until needed, the bivalves were frozen at −4°C to preserve their internal tissues.

$$Condition\,index\ (g/cm^3) = \frac{Tissue\ wet\ weight\ (g)}{Body\ length \times height \times width\ (cm^3)} \times 100\% \quad (1)$$

2.3.2 Particle staining and isolation

Several procedures have been recommended for tissue digestion in literature; however, after review, the ones suggested by Thiele et al. (2019) were adopted. Whole-organism tissues were removed from each bivalve shell using sterilized stainless-steel tools, and immediately placed into separate 100 mL glass Erlenmeyer flasks. No pooling of the samples was conducted to allow for the determination of particles per individual. Tissue digestion was carried out by incubating the removed tissue samples in freshly prepared 10% potassium hydroxide (KOH) (4 times the tissue volume) for 48 h at 60°C within a Precision® Gravity Convection Incubator. After 24 h, 2.5 mL of Rit Dye More® was added into the digestion mixture to stain any synthetic polymers purple for identification. A face wash containing polyethylene (PE) (Neutrogena® Oil-Free Acne Wash) was used as a positive control. Following incubation, each sample was vacuum filtered through a Whatman™ 1001–090 Grade 1, Pore Size, 9 µm qualitative cellulose nitrate filter paper and rinsed with 30% hydrogen peroxide (H_2O_2) to remove any biofilm or fat membrane remnants. Once dried, filter papers were placed into

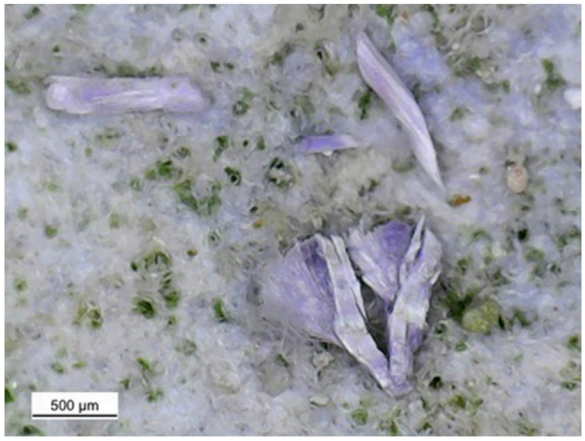

FIGURE 2
Microscopic image of dyed Cyanox®53 fragments collected from Burrard Inlet.

Petri dishes and sealed with Parafilm® M all-purpose tape until inspection.

2.4 Filtrate inspection and ATR-FTIR

Altogether, 21 replicates of sediment and 175 bivalves were evaluated. Filtrates extracted from bivalve samples were visually observed through a digital dissection microscope at ×60 magnification. As suggested in Maes et al. (2017), fluorescent tagged filtrates found in the sediment samples were visually inspected using an orange filter digital dissection microscope (×60 magnification) under blue excitation (420–495 nm). Any white fragmented/frayed particles suspected of being Cyanox®53 were submitted to ATR-FTIR spectroscopy for verification (Figure 2). Infrared spectra between 550 cm^{-1} and 4000 cm^{-1} were measured on a PerkinElmer FTIR Microscope Spotlight 200i, with the focus set to a 10 µm area of the particle using a diamond crystal. Before examination, each filter paper was sprayed with methanol (≥99.5% purity) to prevent loss of particles. Each ATR-FTIR spectra was assigned a polymer identify based on "best match" according to molecular bond vibrations and a "confidence" score ranging from 0% to 100%; a value of ≥70% was deemed as an acceptable level of confidence to account for weathering. Obtained ATR-FTIR spectra were compared with accessible online spectral databases (e.g., SpectraBase™). If a positively matched Cyanox®53 ATR-FTIR spectra was recovered (≥70% confidence score), the sample was reserved for follow-up analyses.

Total numbers of recovered Cyanox®53 and MP per bivalve and sediment sample were converted to number of particles per g of wet tissue weight (particles/g ww) and number of particles per kg of sediment dry weight (particles/kg dw), respectively. Biota-sediment accumulation factor (BSAF) ratios were calculated using an adapted formula from Alava (2021) to assess the bioaccumulation potentials of mean Cyanox®53 and/or MP concentrations in the bivalve tissue against that deposited in the sediments on a kg weight basis for each site (Equation 2). If the BSAF ratio was >1, bioaccumulation was deemed probable, but if the BSAF ratio was ≤1, then a lack of bioaccumulation was concluded.

$$BSAF = \frac{Mean\ particle\ concentration\ in\ biota\ (particles/kg\ ww)}{Mean\ particle\ concentration\ in\ sediment\ (particles/kg\ dw)} \quad (2)$$

2.5 Structural identification of Cyanox®53

2.5.1 Single-batch equilibrium leaching

Numerous studies have examined the leaching of additives from plastic pollution, with the most commonly used method being single-batch equilibrium leaching (Bridson et al., 2021). Adopting a similar approach, single-batch equilibrium leaching was carried out by placing 1 mg of the recovered Cyanox®53 and Cyanox®53-polyvinylidene fluoride (PVDF) particles into two separate 1 mL Pyrex® glass test tubes and immersing them in 200 µL of 99.9% deuterated dimethyl sulfoxide (DMSO-d_6) (supplied by the Chemistry Department at Simon Fraser University) for 10 days without exchange of the solution. Samples were incubated in a Sybron Thermolyne™ Dri-bath heat block at 70°C while being shaken at 200 rpm on a Fisher Scientific™ Multi-Platform shaker to promote maximum partitioning.

2.5.2 ^{1}H-NMR and ESI-LC/MS

A calibration standard of tetramethylsilane (0.01%) was used to evaluate the signal-to-noise ratio (between the 3–7 ppm range) and to ensure a linear calibration curve was produced. ^{1}H-NMR measurements of the leached Cyanox®53-PVDF and Cyanox®53 samples were recorded on a Bruker Avance II 600 MHz spectrometer, equipped with a cryoprobe. Both ^{1}H-NMR spectra were acquired with 1024 scans at 298 K, between −1 ppm and 14 ppm, and referenced to the residual DMSO resonance at 2.50 ppm. As references, 10 mg of dry extract 2.2′-methylenebis (4-ethyl-6-tert-butylphenol) (≥98.0% purity), 2.2′-methylenebis (6-tert-butyl-4-methylphenol) (≥99.0% purity), 4.4′-isopropylidenediphenol (≥99.0% purity), respectively, were dissolved into 2 mL of 99.9% DMSO-d_6 separately. Their ^{1}H-NMR spectra were also acquired on a Bruker Avance II 600 MHz spectrometer equipped with a cryoprobe at 298 K, between −1 ppm and 14 ppm, with a relaxation delay of 5 for 128 scans. The chromatographic separation was achieved on an Agilent 6210 TOF LC/MS using ESI-MS. Each sample concurrently ran on a C_{18} LC gradient from water-acetonitrile (0%–100%) for 8 min. Scans were ran in positive mode from 100 to 1500 m/z; a flow rate of 100 µL/min and an injection volume of 10 µL was used. All obtained m/z values were background corrected to account for the water-acetonitrile mobile phase (~18 Da). Procedural blanks were processed and analyzed alongside NMR and MS analyses to account for potential ambient or processing contamination of samples. All collected NMR and MS data were analyzed in MestReNova, Version 14.2.1 (MestReNova, 2021).

2.6 Modeling approach

2.6.1 Bioenergetic model

To assess the potential risk of Cyanox®53 if it were to leach from its solid form, the amount of Cyanox®53 ingested daily by an

overwintering sea duck species, the surf scoter (*M. perspicillata),* was estimated on a particles/kg body weight/day basis using the model from Bendell (2011) (Equation 3). Full details of the modeling approach can be found in Bendell (2011). In brief, the number of MPs ingested by a surf scoter each day can be estimated if one knows i) the daily kJ energy required by a surf scoter, ii) the kJ content of a varnish clam, iii) the number of clams ingested per day by a surf scoter, iv) the average number of MPs per clam, and v) the average size of a surf scoter. An average size of a surf scoter is ca. 1 kg, therefore the equation used to estimate MP ingestion would be:

$$\begin{aligned} &particles\ Cyanox^{®}53\ ingested/kg\ body\ weight/day \\ &= \left(\left(\frac{daily\ \frac{kJ}{day}\ required}{kJ\ per\ varnish\ clam} \right) \times (g\ ww\ of\ varnish\ clam) \right) \\ &\quad \times \left(Cyanox^{®}53\ particles/g\ ww \right) \end{aligned} \tag{3}$$

Using this approach we calculated the average, minimum and maximum number of particles of Cyanox®53 that would be ingested on a daily basis, then as the total amount ingested over a 4-month overwintering period. It should be noted that these are estimates that assume a constant daily rate of ingestion. Energetic requirements of the scoter can vary daily based on prey availability with energy expenditure inversely related to amounts of prey available, and external factors such as weather (i.e., temperature variation and storm events).

2.6.2 QSAR

Data collected from ATR-FTIR, ^{1}H-NMR, and ESI-LC/MS analyses, and the documented structures of the purchased reference chemicals (see section 2.5.2), were used to propose a chemical structure for Cyanox®53. Conversion of the proposed structure into Simplified Molecular Input Line Entry System (SMILES) notation was conducted in JChem for Office, Version 21.11.10 (Cheminformatics Software, 2021). Estimated physical/chemical and environmental fate properties of Cyanox®53 were predicted using the Windows®-based screening level tool Estimation Program Interface (EPI) Suite™ Version 4.11 (US EPA, 2021). Potential acute and chronic toxicity values of Cyanox®53 to aquatic organism surrogates, such as fish, *Daphnia magna*, and green algae, were predicted with ECOSAR™ embedded in EPI SUITE, 2021. If the log K_{ow} of Cyanox®53 was greater than the endpoint specific cut-off established, then no effects at saturation (NES) were expected for that endpoint. Additionally, if the predicted effect level exceeded the water solubility of Cyanox®53 by ≥ 10-fold, then NES was reported. Default settings were used to run the QSAR software, and no changes were made to the developers' programming.

3 Results

3.1 Environmental survey

3.1.1 Sediment

Cyanox®53 concentrations ranging from 1 to 5 particles/kg dw per site were detected in surface sediments from AP, BMP, EB, and SB. The highest mean concentration of Cyanox®53 was observed at both AP (1.67 ± 0.88 particles/kg dw) and EB (1.67 ± 1.67 particles/kg dw), while the lowest mean concentration was found at SB (0.33 ± 0.33 particles/kg dw) (Figure 3). MP concentrations ranging from 4 to 18 particles/kg dw per site were also detected in surface sediments from all seven sites. The highest mean concentration of MPs was reported at EB (15.7 ± 0.33 particles/kg dw) while the lowest mean concentration was found at J (6.67 ± 1.33 particles/kg dw) (Figure 3).

3.1.2 Bivalves

Bivalve biometric data did not require transformation for any of the seven bivalve populations as shell width (cm), height (cm), length (cm), volume (cm^3), and tissue weight (g) were all found to be normally distributed following frequency distribution, normal quartile-quantile plotting, and Shapiro-Wilk normality tests ($p > 0.05$). A summary of mean biometric data and condition indices (g/cm^3) for each site is provided in the Supplementary Material. Cyanox®53 concentrations were recovered in bivalves from four out of the seven surveyed sites (AP, BMP, EB, and SB). Between 4% and 44% of the individuals possessed Cyanox®53 within their digestive tract. The highest mean concentration of Cyanox®53 was observed at AP (0.25 ± 0.21 particles/g ww), while the lowest was detected at BMP (0.0048 ± 0.0048 particles/g ww) (Figure 4). MPs accumulated within the digestive tracts of almost all sampled bivalves, resulting in frequency levels ranging between 80% and 100%. The highest mean concentration of MPs was observed at AP (0.80 ± 0.21 particles/g ww), while the lowest was recorded at EB (0.19 ± 0.040 particles/g ww) (Figure 4).

3.1.3 BSAF ratios

Collectively, particle concentrations accumulated in bivalve tissue exceeded concentrations deposited in surface sediment samples. Ratios ranging from 0 to 147 were calculated for Cyanox®53, while MPs demonstrated ratios between 12.2 and 88.7. A summary of these ratios is shown in Table 1.

3.1.4 Bioenergetic modeling

Estimates (mean with standard errors) of the daily amounts of Cyanox®53 particles ingested by the surf scoter are presented in Table 2. Ingestion rates are site dependent with birds foraging at AP ingesting the greatest amounts as opposed to birds that forage at BMP.

3.2 Chemical analysis

3.2.1 ATR-FTIR

Ninety-two fragments (<5 mm) positively associated with Cyanox®53 at a ≥70% confidence were recovered from bivalve and sediment samples. Most of the fragments were identified as a chemical mixture of Cyanox®53 with either PVDF (49) or PE (8), while the remaining 35 fragments were assigned the chemical identity of Cyanox®53. ATR-FTIR spectrums of field-collected fragments are provided in the Supplementary Material. The following functional groups based on obtained ATR-FTIR sorption peaks were identified for Cyanox®53: hydroxyl alcohols (3600–3300 cm^{-1} and 950–800 cm^{-1}), in-plane, out-of-plane and radical alkyls (3450–3300 cm^{-1}, 2850–2750 cm^{-1}, 1250–1000 cm^{-1}, and 790–600 cm^{-1}), and two aromatic rings (3100–2750 cm^{-1}, 2600–2450 cm^{-1}, 1800–1700 cm^{-1}, and 1600–1250 cm^{-1}).

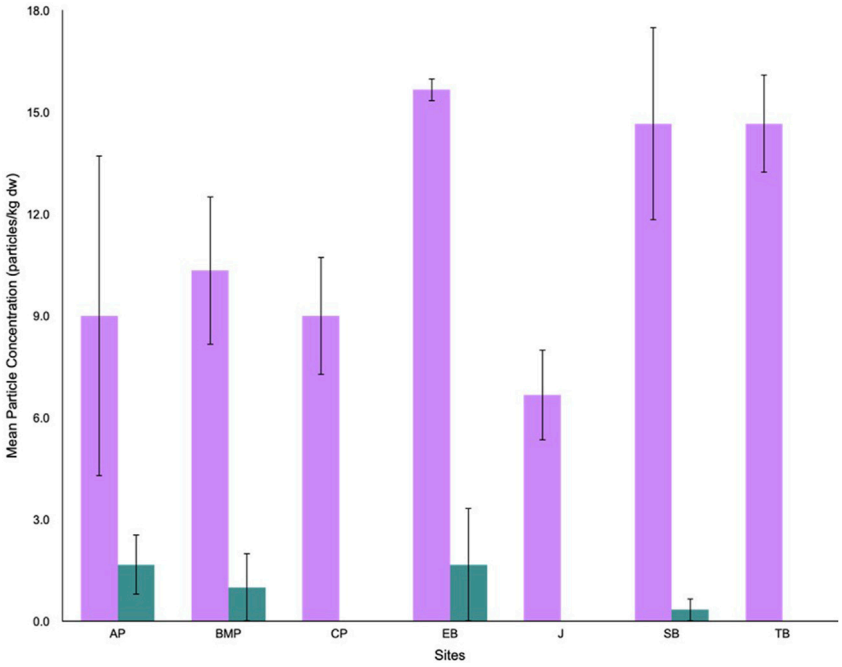

FIGURE 3
Mean particle concentrations (particles/kg dw) of microplastics (purple) and Cyanox®53 (teal) recovered from surface sediments at each sampling site in Burrard Inlet.

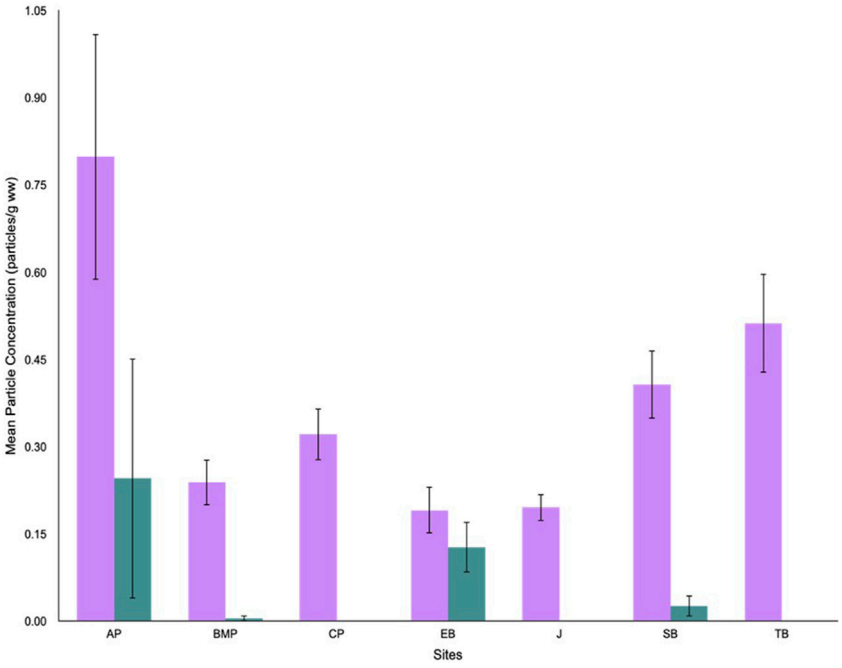

FIGURE 4
Mean particle concentrations (particles/g ww) of microplastics (purple) and Cyanox®53 (teal) recovered from varnish clam at each sampling site in Burrard Inlet.

TABLE 1 Biota-sediment accumulation factor ratios based on mean Cyanox®53 and microplastic concentrations recovered from sampled bivalves and sediments.

Site	Cyanox®53	Microplastics
AP	147	88.7
BMP	4.8	23.1
CP	-	35.7
EB	76.3	12.2
J	-	29.3
SB	77.7	27.7
TB	-	34.9

3.2.2 ESI-LC/MS

Reference chemicals bisphenol A (BPA), Cyanox®2246, and Cyanox®425 returned m/z values that aligned with literature measurements. In the Cyanox®53 sample five distinct intensity peaks were observed at retention times 5.31, 6.06, 6.75, 7.14, and 7.46 min. Similarly, four individual intensity peaks were observed at retention times 5.26, 6.75, 7.16, and 7.46 min in the Cyanox®53-PVDF sample. No cross-contamination by PVDF was observed in the Cyanox®53-PVDF spectra. Through the process of elimination, the m/z value of 358.4 Da was deemed the authentic molecular mass for Cyanox®53. All obtained MS spectra are provided in the Supplementary Material.

3.2.3 ^{1}H-NMR

Reference chemicals BPA, Cyanox®2246, and Cyanox®425 produced ^{1}H-NMR spectra that aligned with literature measurements. Similar chemical shifts and integral values were obtained in the Cyanox®53 and Cyanox®53-PVDF samples. Peaks appeared at 8.47, 5.91, 5.86, 1.24, and 0.86 ppm, and corresponded to the integral values of 1.00 (hydroxyl), 1.08 (hydrogen), 1.08 (hydrogen), 9.52 (*tert*-butyl) and 3.36 (methyl), respectively. No cross-contamination by PVDF was observed in the Cyanox®53-PVDF spectra. Any peaks corresponding to water (3.50 ppm) and DMSO-d_6 (2.50 ppm) were considered as negligible background. All obtained ^{1}H-NMR spectra, and ^{1}H-NMR measurements and structural assignments for each chemical, are provided in the Supplementary Material.

3.3 Structure and QSAR modeling

3.3.1 Proposed structure

Collected ATR-FTIR, ^{1}H-NMR, and ESI-LC/MS data, and the reference of documented structures for BPA, Cyanox®2246, and Cyanox®425, allowed for the elucidation of a feasible chemical formulation for Cyanox®53 as 2.2′-thiobis (3-methyl-5-tert-butylphenol) or $C_{22}H_{30}O_2S$. Application of JChem Office directly translated 2.2′-thiobis (3-methyl-5-tert-butylphenol) to the SMILES notation of CC1 = C(SC2 = C(C)C=C(C=C2O) C(C)(C)C)C(O) = CC(=C1)C(C)(C)C. A chemical search by said SMILES notation in PubChem, ChemSpider, and CompTox Dashboard databases, however, returned no known CAS registry number. The proposed structure of Cyanox®53 along with the chemical structures of BPA, Cyanox®2246, and Cyanox®425 are shown in Figure 5.

3.3.2 EPI Suite™

Predictions of physical/chemical environmental fate and aquatic toxicity properties were made for Cyanox®53 (Table 3). Based on the atom/fragment contribution, a log K_{ow} value of 8.24 was estimated by KOWWIN™. Melting and boiling points for Cyanox®53 were estimated at 196°C and 466°C, respectively by MPBPVP™. Water solubility estimates (at 25 °C) of 0.00191 mg/L (based on log K_{ow}) and 0.0568 mg/L (based on chemical fragments) were calculated by

TABLE 2 Mean particle concentrations with standard error, frequency of Cyanox®53 recovered in bivalves and estimated number of particles ingested on a daily basis for male and female surf scoters foraging on varnish clams (from Bendell, 2011).

Site	n	Individuals with Cyanox®53	Total Cyanox®53 recovered	Number of particles recovered/ Individual	Mean Cyanox®53 concentration (particles/g ww)	Mean number of particles ingested/day/ scoter	
						Male	*Female*
AP	25	6	29	1.16	0.25 ± 0.21	198	210
BMP	25	1	1	0.04	$4.78E^{-3}$ ± $4.78E^{-3}$	171	181
CP	25	0	0	0	0.00 ± 0.00	0	0
EB	25	11	44	1.76	0.13 ± 0.04	684	724
J	25	0	0	0	0.00 ± 0.00	0	0
SB	25	2	4	0.16	0.03 ± 0.02	342	362
TB	25	0	0	0	0.00 ± 0.00	0	0
Mean with SE		2.85 ± 1.58	11.1 ± 6.7	0.44 ± 0.27	-	200/ day ±94.5	210/ day ±100.1

The total number of clams/total number of particles = 0.46 particles/clam for an overall ingestion rate of 78 and 83 particles/day/scoter for males and females, respectively.

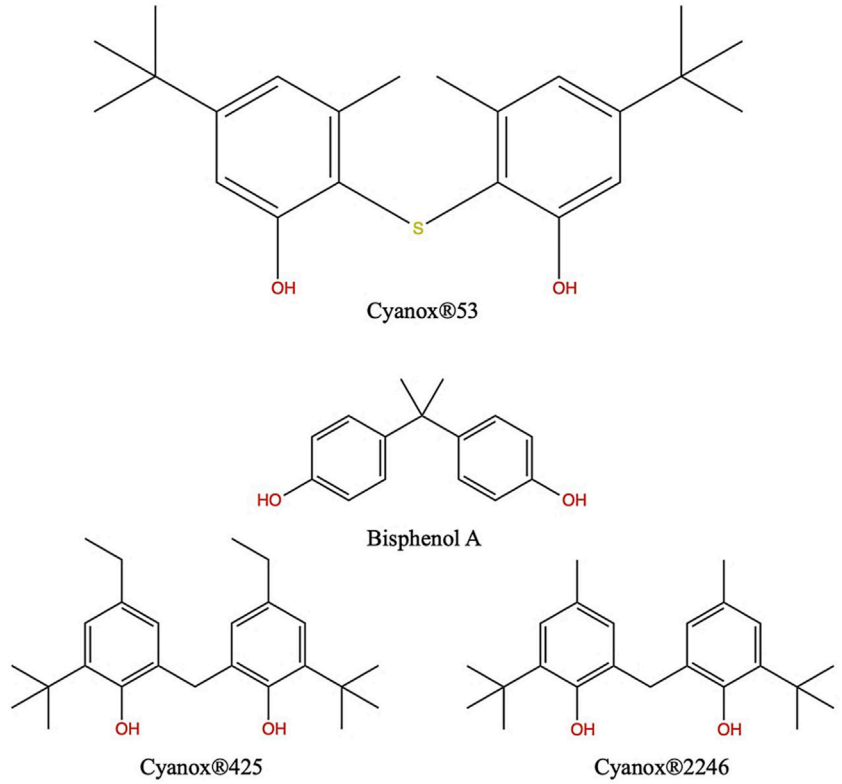

FIGURE 5
Proposed molecular structure of Cyanox®53 compared to reference chemicals BPA, Cyanox®2246, and Cyanox®425.

WSKOWWIN™ and WATERNT™, respectively. BCF and BAF estimations in fish were acquired with BCFBAF™. The following estimates were obtained: BCF = 3,270 L/kg wet-wt (regression method based on K_{ow}), BCF = 28.22 L/kg wet-wt (Arnot-Gobas upper trophic method), and BAF = 308 L/kg wet-wt (Arnot-Gobas upper trophic method). A biotransformation half-life in fish equivalent to 2.5 days (normalized to a 10 g fish) was also predicted. An ultimate survey and primary survey timeframe of 1.946 (months) and 2.966 (weeks), respectively, and a MITI linear model value of 0.0274 were estimated in the evaluation of rapid biodegradability. Accordingly, the BIOWIN™ model criteria for rapid biodegradation was not satisfied. Moreover, log K_{oc} estimates of 6.30 (based on MCI method) and 5.82 (based on K_{ow}) were obtained by KOCWIN™. Partitioning half-life times of Cyanox®53 in air, water, soil, and sediment under steady state conditions were estimated to be 1.27 h, 60 d, 120 d, and 540 d, respectively. An estimated maximum half-life persistence of 189 d was computed.

ECOSAR™ was used to estimate acute toxicity endpoints at 50% lethal concentration (LC_{50}) for fish and *D. magna*, and 50% effective concentration (EC_{50}) for green algae. For fish, a simulated 96 h exposure predicted a LC_{50} value of 0.0040 mg/L with a log K_{ow} maximum of 7.0. A LC_{50} value of 0.0020 mg/L with a log K_{ow} maximum of 5.5 was predicted for *D. magna* in a simulated 48 h exposure. A 96 h exposure manifested a EC_{50} value of 0.058 mg/L with a maximum log K_{ow} of 6.4 for green algae. Chronic toxicity effect endpoints were also predicted for fish, *D. magna* and green algae based on a geometric mean between the lowest observed effects concentration (LOEC) and no observed effect concentration (NOEC) of the study. A maximum log K_{ow} cut-off was set to 8.0 (indicative of a poorly soluble chemical) as a baseline for comparison in fish, *D. magna* and green algae. Chronic effect concentrations of 0.00078 mg/L, 0.00062 mg/L, and 0.020 mg/L were predicted for fish, *D. magna*, and green algae, respectively.

4 Discussion

4.1 Environmental survey

Our study recovered Cyanox®53 concentrations in the surface sediments and varnish clam of several sites in Burrard Inlet, BC. As predicted, Cyanox®53 concentrations were found to be the most abundant near the First Narrows strait (mouth of outer and inner harbor) where the majority of the Port of Vancouver is centralized. The highest levels were presented at AP and EB, with no significant difference being noted between these sites in either sampling medium. These results coincide with recent reports of Cyanox®53 concentrations bioaccumulating in marine coastal sediments and bivalves (Knutsen et al., 2019; Bendell et al., 2020; Achiluzzi, 2022). Like the previous surveys, a key characteristic of the Cyanox®53 particles in this study was their disassociation from its inert carrier and abnormal presentation as a white, fragmented entity. It is unknown how or why this alleged powdered additive

TABLE 3 Comprehensive summary of estimated physiochemical and ecotoxicity of Cyanox®53.

Pluggin	Endpoint	Prediction
KOWWIN™	Log K_{ow}	8.24
MPBPVP™	Melting and boiling points	196°C, 466°C
WSKOWWIN™, WATERNT™	Water solubility (at 25 °C)	0.00191 mg/L (K_{ow} method), 0.0568 mg/L (chemical fragments method)
BCFBAF™	BAF	308 L/kg wet-wt (Arnot-Gobas method)
-	BCF	3,270 L/kg wet-wt (regression), 28.22 L/kg wet-wt (Arnot-Gobas method)
-	Biotransformation half-life in fish	2.5 days (normalized to a 10 g fish)
BIOWIN™	Biodegradability prediction	Not readily biodegradability
KOCWIN™	Log K_{oc}	6.30 (MCI method), 5.82 (K_{ow} method)
LEV3EPI™	Partitioning half-life in air, water, soil, and sediment; persistence time (max half-life in environment)	1.27 h (air), 60 d (water), 120 d (soil), and 540 d (sediment); 189 d (persistence time)
ECOSAR™	Fish, LC_{50} (96 h)	0.0040 mg/L, max log K_{ow} at 7.0
-	Daphnia magna, LC_{50} (48 h)	0.0020 mg/L, max log K_{ow} at 5.5
-	Green algae, EC_{50} (96 h)	0.058 mg/L, max log K_{ow} at 6.4
-	Fish, chronic	0.00078 mg/L, max log K_{ow} at 8.0
-	Daphnia magna, chronic	0.00062 mg/L, max log K_{ow} at 8.0
-	Green algae, chronic	0.020 mg/L, max log K_{ow} at 8.0

continues to emerge as a fibrous solid in the environment. If recent environmental surveys are considered, however, a trend of Cyanox®53 particles appearing in areas with intense shoreline alterations and maritime activities manifests, in which case, one can speculate that a link exists between Cyanox®53 particles and anthropogenic pressures. Additional backing to this surmise comes from the fact that Cyanox®53 can be integrated as a thermal stabilizer into rubbers, cable resins, coating liners of PE wiring and thermally insulated piping, and that some Cyanox® variants can provide processing and thermal stability in rubbers products, acrylics, and polyolefins marketed to the construction industry (Flick, 2001; Cruce et al., 2014; Bolgar et al., 2016; Solvay, 2020). Alternatively, Cyanox®53 could be potentially associated with pyrotechnics.

Though it depends on scale of the event, fireworks are a significant source of MP debris that contribute to atmospheric, terrestrial, freshwater, and marine pollution (Devereux et al., 2022). On average a pyrotechnic mixture will contain roughly 10% of a polymeric binder, either made from a natural (e.g., starch), synthetic (e.g., novolac), or synthetic polymer (e.g., polyvinyl chloride) material (Naik and Patil, 2015; Toader et al., 2017; Devereux et al., 2022). In certain circumstances, fireworks may also include plastic attachments, such as a mortar and tube at the bottom to boost their momentum at launch, or a cone at the top to aid their flight pattern, leading to the discharge of additional plastic debris upon denotation (Naik and Patil, 2015; Devereux et al., 2022). Other toxic substances, metals, airborne particulates, and chemical pollutants have also been shown to accumulate near the fireworks display areas following detonation (Devereux et al., 2022). In Vancouver, BC, several fireworks events are held annually off-shore in EB, including New Year's, Canada Day, Halloween, and most notoriously the three-night international fireworks competition "Honda Celebration of Light". Incidentally, EB and AP were also two locations in this present study with the highest recovered Cyanox®53 particle concentrations. Considering this, along with the fact that a prominent number of the Cyanox®53 fragments possessed a shrapnel-like appearance and chemical association with PE or PVDF (two thermoplastics commonly used in packaging and industrial applications), it is plausible that Cyanox®53 could be incorporated as an additive either directly into the pyrotechnic composition, or indirectly in the outer plastic attachments if equipped, to offer stability. To explore these speculations further investigation is warranted.

A high abundance of MPs (i.e., high-density polyethylene, low-density polyethylene, polypropylene, polystyrene, and polyvinyl chloride) was also recovered in sediments and clam tissue, with concentrations being more abundantly prevalent than Cyanox®53 at all assessed sites in both sampling mediums. Expressed BSAF ratios demonstrated that bivalves accumulated higher mean particle concentrations, more frequently MPs (80%–100%), in their guts and gills than the intertidal sediment samples. This finding is unsurprising given that sediments act as a sink for negatively buoyant MPs, meaning bivalves, to varying degrees, will inevitably accumulate suspended MPs as they pull particles (i.e., diatoms, bacteria, phytoplankton, etc.) into their incurrent siphons from the water column, or collect organic detritus from the sediment with their foot (Gillespie et al., 1999; Gillespie et al., 2001; Hidalgo-Ruz et al., 2012; Cho et al., 2021; Ding et al., 2021). It is because of this that bivalve species (e.g., *Venerupis philippinarum*, *Siliqua patula*, *M. edulis*, and *Crassostrea gigas*) have a history of use

as biomarkers of MPs contamination levels, as well as pollutants, hypoxia, and algal toxins, occupying the water column and surrounding sediments (Baechler et al., 2020; Hermabessiere et al., 2019; Bendell et al., 2020; Cho et al., 2021; Huffman Ringwood, 2021). In the case of this present study, the largely sessile, sediment dwelling varnish clams functioned as suspension and deposit feeders, thus increasing their likelihood of accumulating any marine debris and xenobiotic particles within the water interface and sediment (Gillespie et al., 2001). Ultimately, these results support the notion that opportunistic feeding bivalve species are suitable bioindicators of MP pollution. Furthermore, it demonstrates that opportunistic bivalves could be used as indicators of MP bioaccumulation, perhaps based on the function of retention time and/or elimination rate of the MPs in the bivalve, to support the categorization and screening of potentially bioaccumulative MPs and nanoplastics in bioaccumulation assessments (Alava, 2021).

4.2 Ecological significance

Plastic ingestion has been documented in over 100 species of seabird (Laist, 1997). Examples include Trevail et al. (2015), who studied plastic ingestion in high Arctic Fulmarus glacialis from Svalbard, finding that 87.5% of the 40 sampled fulmars had ingested plastics, averaging 0.08 g or 15.3 ± 5.51 pieces per individual. This challenges the trend of decreasing plastic ingestion with distance from human activity. Regional comparisons showed average plastic ingestion ranging from 0.55 g in the English Channel to approximately 0.2 g in Canada, with over 80% of the populations, except Canada, having plastics in their stomachs. Another example is Provencher et al. (2014) who reviewed marine debris in North Atlantic seabirds, finding that of the 13 species studied, 5 contained ingested marine debris. Plastic ingestion frequency was low in common eiders (1%), but moderate in Leach's storm-petrels, thick-billed murres, and Atlantic puffins (50%). Great shearwaters had the highest prevalence (71%), with a median of 2 plastic pieces per bird and up to 36 pieces in some individuals. Detailed plastics data was available for detailed examination from 11 great shearwaters (range 0–36) and the number of pieces in their GITs was relatively high (two individuals had 36 plastic pieces in their GITs). It was also shown that Northern fulmars had significant ingestion, with 51% of individuals containing plastics. In their review, Provencher et al. (2014) noted that a bird's foraging mode is assumed to play a large role in how much marine debris it ingests. These authors suggested that diving birds, such as auks and sea ducks, are expected to have relatively low levels of plastics because they rarely feed at the water surface where most plastics tend to float. By contrast, surface-feeding birds, represented by great shearwaters, northern fulmars, Arctic terns, and Leach's storm-petrels, are thought to be more susceptible to ingesting plastics with the findings of their review supporting this prediction, i.e., great shearwaters and northern fulmars exhibiting the highest levels of plastic ingestion (71% and 51% occurrence, respectively). Not considered, however, and as shown in our study, is the trophic transfer of MPs from sediments to sediment-ingesting bivalves such as the varnish clam and then to predators such as diving ducks.

Despite their potential hazards, the ingestion and effects of MPs are not fully understood. Yahya et al. (2023) reviewed the occurrence and pathways of MPs in aquatic environments, highlighting a significant gap in understanding their ingestion and effects. The available studies indicate health hazards such as gut leakage, pathogenic bacterial transfer, and increased toxicity, though most research has focused on fish, with limited information on seabirds. Sun et al. (2024) investigated the combined effects of PVC MPs and cadmium on Muscovy ducks, finding that co-exposure induced oxidative stress and fibrosis, disrupted mitochondrial structure and function, and promoted pancreas inflammation and fibrosis.

Recent work by Charlton-Howard et al. (2023) on the Flesh-footed Shearwater from Lord Howe Island provides one of the first studies to demonstrate the effects of solid plastics particles on seabird health. In their study, widespread scar tissue formation and changes to the prevalence of collagen within the stomach of the birds was associated with exposure to plastics (Charlton-Howard et al., 2023). The outcome of these findings was identified as a new plastic induced fibrotic disease, "Plasticosis", a possible disease that the varnish clam eating bird populations could be susceptible to. MPs have also been shown to serve as carriers of viral diseases through adsorption, thus increasing the risk of environmental dissemination of water-borne viruses (Liu et al., 2022).

Burrard Inlet, which is part of the Salish Sea is recognized as one of the world's most important bird areas supporting globally vital bird populations (Bowman et al., 2022). It is especially important to sea ducks of the Pacific Flyway during wintering, staging, spring migration, and molting. Indeed, this site is a major wintering location for 11 species of sea ducks, including Surf Scoter (*Melanitta perspicillata*), White-winged Scoter (*Melanitta deglandi*), Black Scoter (*Melanitta americana*), Bufflehead (*Bucephala albeola*), Common Goldeneye (*Bucephala clangula*), Barrow's Goldeneye (*Bucephala islandica*), all three mergansers (*Mergus* spp.), Long-tailed Duck (*Clangula hyemalis*), and Harlequin Duck (*Histrionicus*) (Bowman et al., 2022). Lewis et al. (2008) and Palm et al. (2012) have noted that varnish clams make up the main diet for the wintering white-winged scoter, although Anderson et al. (2008) note that soft-bodied prey items are also of importance. Recently, Hollenberg and Demers (2017) reported that in addition to sea ducks, shorebirds such as the Black Oystercatcher (*Haematopus bachmani*) forage on varnish clams with little size selectivity.

To our knowledge, there have been no bioenergetic models applied to determine how much MP on a Gram basis is ingested by seabirds. The scoter duck in this case provides an opportunity to do so as: 1) they ingest solely one diet item, the varnish clam, 2) their energetic requirements on a daily basis are known and 3) their behavior with respect to overwintering in a particular area is known. Therefore, as done by Bendell (2011), a bioenergetics model was applied here to assess the amount of Cyanox®53 particles ingested by an overwintering sea duck species, using the surf scoter as a model. In Burrard Inlet, key overwintering months include November, December, January, and February (Bendell and Wan, 2010). Generally, scoters will have an energy intake of 1035 kJ/day and 1100 kJ/day for male and female scoters, respectively (Table 2; Bendell, 2011). Based on values provided by Simmons et al. (2014), a 36 mm varnish clam provides 4.64 kJ of energy; in the current study, the average size of the collected varnish clams was

47 mm, which provides 6.05 kJ of energy. The average number of Cyanox®53 particles recovered from varnish clams across the seven sites was 0.46 particles/clam. Considering these details, a male and female scoter will have ingested 78 and 83 particles of Cyanox®53, respectively, in one day. Over four wintering months, 120 days, male and female scoters will have ingested 9421.8 and 10,086 particles of Cyanox®53, respectively. According to Senathirajah et al. (2021), who estimated the weight of MPs based on size, the mean mass of an individual MP particle ranges from 2.8 to 3.99 g/particle x 10^{-3} for a 0–1 mm particle size; the same size range of Cyanox®53 particles collected in the present study. Using the average of this range, 3.5 g/particle x 10^{-3}, a male and female scoter will have consumed 33 and 35 g, respectively. To put this estimate into perspective, the amount of Cyanox®53 consumed throughout the overwintering period would be the equivalent to a male and female scoter ingesting 5.5 and 6 plastic cutlery forks that weigh 6 g each. We use this analogy to demonstrate the degree to which bivalve-consuming sea ducks are at risk to MPs, such as Cyanox®53, within their primary diet items. It is not the recovery of the number of plastics within the animal at one point of time, but rather the cumulative effects of chronic exposure over a longer period of time. We know of no studies that have tried to assess the toxicological significance of this exposure.

4.3 Persistence, bioaccumulation, toxicity predictions

As mentioned earlier, assessing the toxicity of MPs poses quite a challenge to researchers as they not only constitute a physical obstacle to aquatic life, but also a dynamic vector and potential reservoir of inherent additives and adsorbed hydrophobic anthropogenic contaminants. Within recent decades, QSAR modeling has become favored over conventional *in vivo* experimentation in the identification of physiochemical properties, and toxicological mechanism and structure–activity relationship for dissolved "neat" chemicals (Lapenna et al., 2010; Hayes and Kruger, 2014; OECD, 2022a; OECD, 2022b). Likewise, we applied a similar approach to estimate the potential persistence, bioaccumulation, and toxicity of Cyanox®53 based on a proposed chemical structure, assuming that there is the potential for this compound to leach from the solid phase. The proposed chemical structure for Cyanox®53, which was generated from collected ATR-FTIR, ^{1}H-NMR, and ESI-LC/MS data and the use of BPA, Cyanox®2246, and Cyanox®425 as reference structures, was 2.2′-thiobis (3-methyl-5-tert-butylphenol). From this structure, a log K_{ow} of 8.24 with a low water solubility of 0.00191 mg/L (0.0568 mg/L based on chemical fragments) at 25°C was predicted for Cyanox®53. As a rule, chemicals with high log K_{ow} values (≥5) are deemed to be poorly water soluble and tend to partition to organic matter in soils and sediments within their surroundings (CEPA, 2013; Chi et al., 2018). Provided this, the same outcome would be assumed for Cyanox®53 in its chemical form. Further support to this inference comes from predictions of low mobility in sediment (high log K_{oc} (>5)), low biodegradability (equivalent to 2 months before complete mineralization), partitioning half-lives of 120 and 540 days in soil and sediment, respectively, and a max half-life of 189 days in the environment. Together, these estimates suggest that Cyanox®53 is likely to persist in its chemical form via adsorption to organic matter in the surrounding soils and sediments, and thus have a prolonged fate in the environment.

Bioaccumulation potential is another important parameter to consider in the risk assessment of environmental pollutants. Highly bioaccumulative chemicals are of particular concern to biota given their potential to cause direct adverse health effects (i.e., physiological damage) or indirect toxicity in higher trophic level organisms or/and apex predators at the top of the food web (i.e., biomagnification) regardless of the ambient concentration (CEPA, 2013b). In the simplest model, log K_{ow} can be used to screen the bioaccumulation potential of a chemical based on the assumption that the absorption of an organic substance is driven by its hydrophobicity (European Centre for Ecotoxicology and Toxicology of Chemicals, 2000). However, bioaccumulation potential is not solely dependent on hydrophobicity, rather other underlying mechanisms and physiological properties of an organism can affect the bioconcentration and bioaccumulation potentials of organic substances. For instance, many organic substances can undergo metabolism (biotransformation) and depuration, resulting in a decrease in the bioaccumulation potential of the parent compound (ECETC, 1998; CEPA, 2013a). Moreover, in scenarios where organisms are exposed to very hydrophobic substances (i.e., log K_{ow} > 7.5), a reduction in bioavailability (and therefore bioaccumulation/bioconcentration potentials) can be expected because the chemical will be largely absorbed to any dissolved or particulate organic carbon suspended in the diet (Gobas and Morrison, 2000; Gobas, 2001; Arnot and Gobas, 2003; CEPA, 2013b). By this logic, while the predicted log K_{ow} of Cyanox®53 appears to exceed the threshold set by Environment Canada (log K_{ow} > 5), its measurement cannot provide a definite answer for bioaccumulation potential in its chemical form. Alternatively, BAF and BCF would provide a more realistic interpretation of bioaccumulation potential of a substance (CEPA, 2013a; CEPA, 2013b). In Canada, a criterion of 5000 for BAFs or BCFs are recommended as the threshold to address lipophilic substances with the potential to bioaccumulate and biomagnify (CEPA, 2013a). Concerning Cyanox®53, the predicted BAF (308 L/kg wet-wt Arnot-Gobas method) and BCF (3,270 L/kg wet-wt regression method; 28.22 L/kg wet-wt Arnot-Gobas method) values fell below theses benchmarks, indicating that Cyanox®53 would be unlikely to bioaccumulate or biomagnify up the food-chain in its chemical form. Such implication, however, does not apply to Cyanox®53 in its solid form as it is evident from recent surveys (i.e., Knutsen et al., 2019; Bendell et al., 2020; Achiluzzi, 2022), as well as this current study, that the additive is fully capable of bioaccumulating in sediments and bivalves as a physical particulate. Hence, further investigation into the bioaccumulation potential of Cyanox®53 is recommended. As a future study, one could analyze the retention time and depuration of Cyanox®53 particles accumulated in field-collected varnish clams at different time points (e.g., 1, 2, 4 and 8 d) post collection.

According to the ECOSAR™ model, the proposed chemical structure of this study was found to be too insufficiently water soluble to elicit toxicological effects at saturation in the acute and chronic exposure scenarios for fish, *D. magna*, or green algae when the predicted log K_{ow} of 8.24 was used as the determinant. However, when the predicted water solubility values were applied as the determinants of toxicity, different results were seen. Specifically,

if the predicted water solubility of 0.00191 mg/L (based on the predicted log K_{ow}) was used for comparison, potential toxicological effects were simulated in the acute and chronic exposure scenarios for *D. magna* and fish surrogates, with NES being noted in the green algae. But, if the predicted water solubility of 0.0568 mg/L (based on chemical fragments) was used, potential toxicological effects were observed in acute and chronic exposure scenarios for *D. magna*, fish, and green algae. Clearly, there is a discrepancy between the predicted log K_{ow} and water solubility values. It is because of this disconnect that a definitive answer cannot be given on the toxicological effects of Cyanox®53 based on the EPI Suite™ results.

The likelihood that an excessive amount of Cyanox®53 particles would leach out a high enough concentration to induce significant toxicity acutely before being discharged is considerably low. Moreover, given that frank toxicity was not observed in the varnish clams containing Cyanox®53 particles in this study, the potential of Cyanox®53 causing toxicological effects appears to decrease. All the same, it cannot be said for certain that a toxicological effect would not have eventually taken place on some level in the clams from chronic exposure. Indeed, rather than exert acute toxicity, perhaps the hazardous nature of Cyanox®53 particles stem from their ability to bioaccumulate as a solid chronically and potential to cause physical harm at the organ level, either directly in an organism or indirectly when predatory species unknowingly ingest contaminated prey (i.e., trophic transfer) (Gallo et al., 2018; Nelms et al., 2019). The only way to verify this theory would be to perform chronic *in vitro* or *in vivo* toxicity tests, such as Organisation for Economic Co-operation and Development (OECD) test guidelines No. 201, 204, 210, 211, or 230, with Cyanox®53 particles (OECD, 1984; OECD, 2009; OECD, 2011; OECD, 2012; OECD, 2013).

4.4 EPI Suite™ limitations

Although QSAR analyses have been deemed an accepted method in environmental risk screening, different levels of uncertainty and variability exist within each model and measured parameter. For instance, it is known that log K_{ow} predictions in EPI Suite™ are derived from experimental K_{ow} values of the nearest chemical analogs (Do et al., 2022). Because of this, it increases the probability of inaccurately predicting certain descriptors for a chemical. The simple solution to this issue would be to compare the predicted results to experimental data collected in model-based or laboratory-based research for verification. However, in the case of Cyanox®53, there are no model-based or experimental data for comparison in the literature, therefore making it very challenging to decisively measure its toxicological significance. Coupling this with the fact that the predictions seen in this present study, albeit valuable, are merely based on a feasible structure, the likelihood that uncertainty may exist in the predictions from EPI Suite™ arises. Even so, it should not be implied that the results observed in this study are completely invaluable or inaccurate. Instead, these results represent a baseline for comparison in future studies and should be validated with OECD test guidelines like No. 105, 107, 117, 123, and 301 (OECD, 1992; OECD, 1995a; OECD, 1995b; OECD, 2022a; OECD, 2022b; OECD, 2024).

Another plausible explanation as to why the toxicological predictions of this study were inconclusive could be related to the assumptions of EPI Suite™, namely, the assumption that all compounds exist as neatly dissolved chemicals. At present, most QSAR models are developed specifically for low molecular weight organic compounds, making it quite challenging to evaluate and mitigate the potential environmental impacts of MPs and associated additives (Brunning et al., 2022). In EPI Suite™, its chemical domain of applicability does not cover the full range of substances, nor does it currently produce accurate estimates for inorganic substances, organometallic substances, some ionizable organic compound, large molecular weight (>500 Da) substances, nanomaterials, perfluorinated and other halogenated compounds, compounds without functional groups, and compounds containing significant organic functional groups (Card et al., 2017). When it comes to Cyanox®53, it evidently does not adhere to the above chemical profiles, thus any predictions procured for Cyanox®53 falsely assumed that it existed as a dissolved chemical, rather than a solid particle. Such a misinterpretation can reduce the overall accuracy and predictive powers of a QSAR model. Not to mention, in assuming that Cyanox®53 only exists as a dissolved chemical in the environment, it neglects the possibility that Cyanox®53 particles could bioaccumulate within the tissue of an organism as a solid particulate (as seen in this study) and elicit eventual toxicity through chronic exposure. Concepts such as BCF, which assume chemicals move via passive diffusion, are not applicable to nanoparticles and MPs since their uptake is dependent on several parameters (including size, shape, volume, density, roughness, and polymer composition) and processes like the retention time and/or elimination rate in an organism, rather than the equilibrium partitioning (Alava, 2021; Brunning et al., 2022). To correct this oversight, development of QSARs that consider the influence of different parameters of MP fate, transport, physiochemical properties, and toxicity in the environment is required. Furthermore, robust data collection and research into relevant properties, such as the leachability and retention time of accumulated MPs and alike particles in biota, is warranted.

5 Conclusion

Given the lack of admissions on Cyanox®53, the evidence uncovered in this work shows promise for forthcoming analyses. It was only recently that the presence of Cyanox®53 in the environment had been documented, making this study one of the first to recover particle concentrations in sediments and bivalves from Burrard Inlet, BC. This is also the first study to provide ecological relevance and QSAR predictions of persistence, bioaccumulation, and toxicity for Cyanox®53. Our findings indicate that varnish clams collected from Burrard Inlet accumulate on average 0.46 particles of Cyanox®53/clam, and based on bioenergetic modeling, results in surf scoters potentially consuming 78 (for males) to 83 (for females) pieces of Cyanox®53 daily from foraged varnish clams. Like other bisphenol-related compounds, we hypothesized Cyanox®53 potentially possessed some degree of toxicity towards aquatic organisms and/or seabirds. EPI Suite™ predicted Cyanox®53 to be persistent, however, unlikely to bioaccumulate

as a "traditional" chemical. Furthermore, the estimation of potential acute and chronic toxicity of Cyanox®53 to aquatic organism surrogates, such as fish, *D. magna*, and green algae, was inconclusive. Currently, QSAR models, such as those in EPI Suite™, function on the assumption that organic compounds exist as neatly dissolved chemicals in the environment, and thus can only provide accurate predictions for "traditional" chemicals. This assumption ultimately became a limitation in this study and further demonstrates that we have yet to develop the tools necessary to place an adequate risk or hazard on MPs and associated compounds within aquatic (and terrestrial) ecosystems. Still, the predictions estimated in this present study hold value as they represent a baseline for comparison in future works. If a definitive answer is ever to be obtained concerning the risk of Cyanox®53, however, consideration needs to be given to the development of polymer QSARs. In addition, further investigation into its potential toxicity, leachability, and retention time as a physical particle in organisms is required, most likely through *in vitro* and *in vivo* testing. In bridging these research gaps, it would offer unprecedented opportunities for further upscaling MP data collection, aid in the establishment of key parameters for inter-study or regulatory, and determine the degree to which MPs, such as Cyanox®53, could impact sea ducks and higher trophic predators within marine ecosystems.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions statement

SR: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. LB: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1439573/full#supplementary-material

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