

Occurrence of 6PPD-quinone in cold-climate urban runoff and acute toxicity to four fishes of commercial, recreational and aboriginal relevance

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Contaminants in urban runoff



RESEARCH

ECOTOXICOLOGY

A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon

Zhenyu Tian^{1,2}, Haoqi Zhao³, Katherine T. Peter^{1,2}, Melissa Gonzalez^{1,2}, Jill Wetzel⁴, Christopher Wu^{1,2},

- N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has become a chemical of high concern in stormwater runoff
- Coho salmon were shown to be exceptionally sensitive to 6PPD-quinone (revised $LC_{50} = 0.095 \mu\text{g/L}$) (Tian et al. 2022) -> Suspected to be responsible for mass coho die offs on US west coast
- Other fish species as well as invertebrates tested to date were insensitive (no effects up to water solubility of $\sim 50 \mu\text{g/L}$; Hiki et al. 2021)
- Environmental concentrations of 6PPD-quinone in stormwater frequently exceed the toxicity threshold for coho salmon after runoff events

Research objectives

- Characterize the **occurrences of five compounds released from tire rubber**, including the recently discovered transformation product 6PPD-quinone, **in stormwater** and snowmelt runoff in a semiarid, cold-climate city.
- Investigate the **acute toxicity of 6PPD-quinone across four fishes** of commercial, cultural, and ecological importance to North America: rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), Arctic char (*Salvelinus alpinus*), and white sturgeon (*Acipenser transmontanus*).

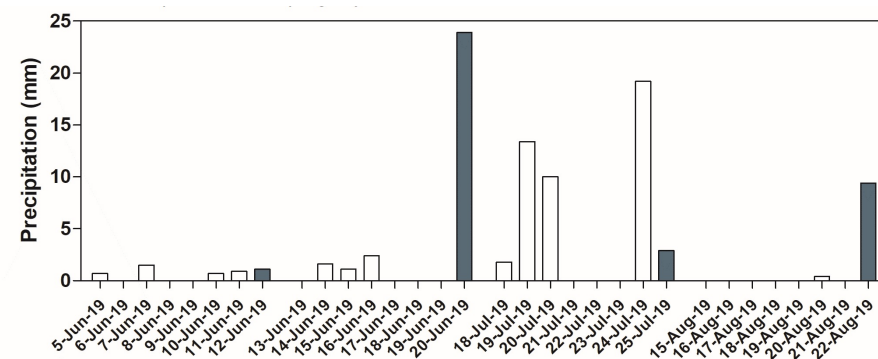


Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff



Study Area: City of Saskatoon

- Located in central Saskatchewan, Canada (52, -106)
- Area of 228 km², population of approx. 300,000 (Statistics Canada 2017)
- Average of 355 mm of precipitation a year, of which 50-100 mm falls between November and March (typically as snow)



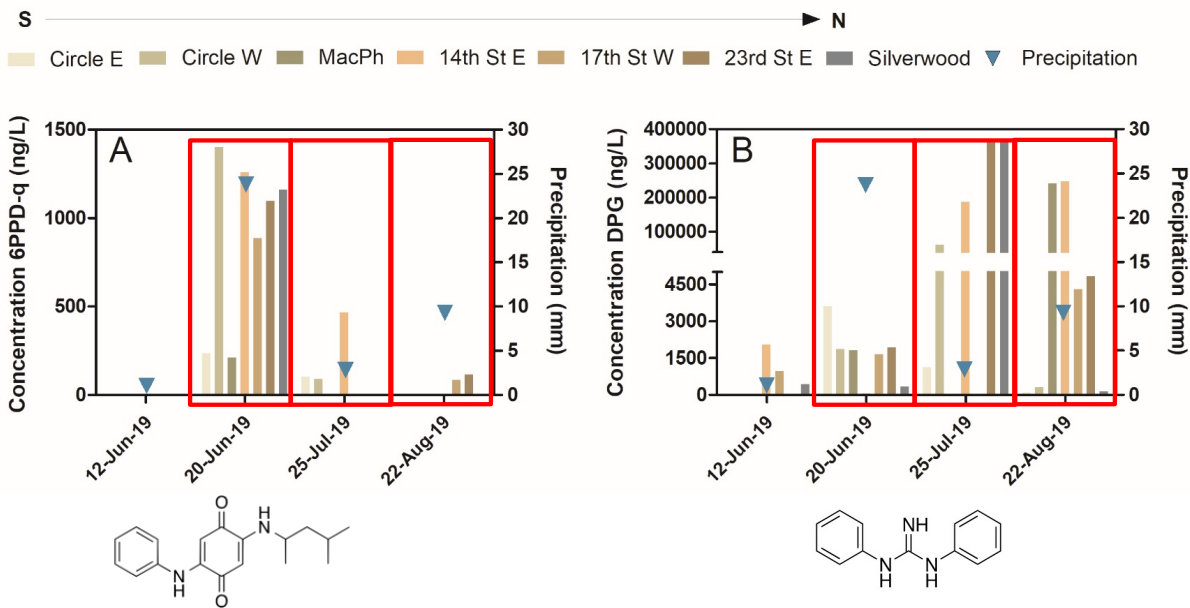
Date	Rainfall (mm)	Antecedent Dry Days	Antecedent Rainfall (mm)
June 12	1.1	33	2.4
June 20	23.9	8	1.1
July 25	22.1	4	13.4
August 22	9.4	20	7.0

Sample analysis and extraction

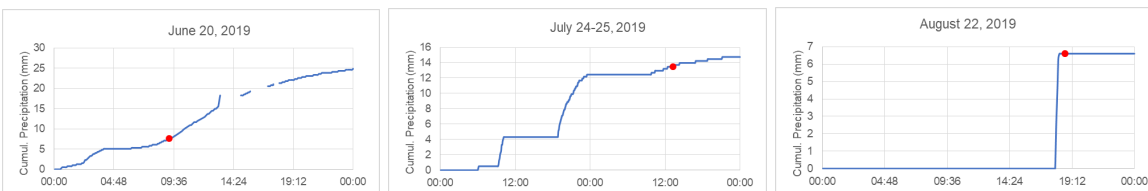
- Tire-rubber derived compounds studied:
 - a) N,N'-diphenylguanidine (DPG)
 - b) N,N-Dicyclohexylmethylamine (DCA)
 - c) N,N'-Dicyclohexylurea (DCU)
 - d) 1-Cyclohexyl-3-phenylurea (CPU)
 - e) 6PPD-quinone
- Target compounds identified initially by suspect screening workflow with LC-Orbitrap HRMS
- Authentic standards confirmed identifications and used for semi-quantification
- All samples extracted by solid phase extraction (500 mL)
- Direct injection used to quantify samples with high DPG concentrations



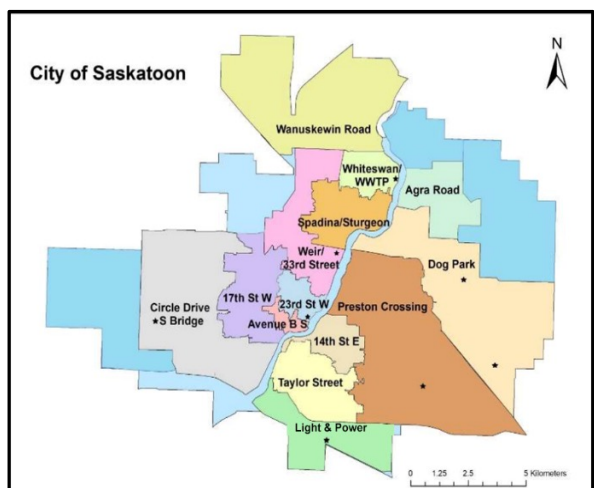
Stormwater concentrations



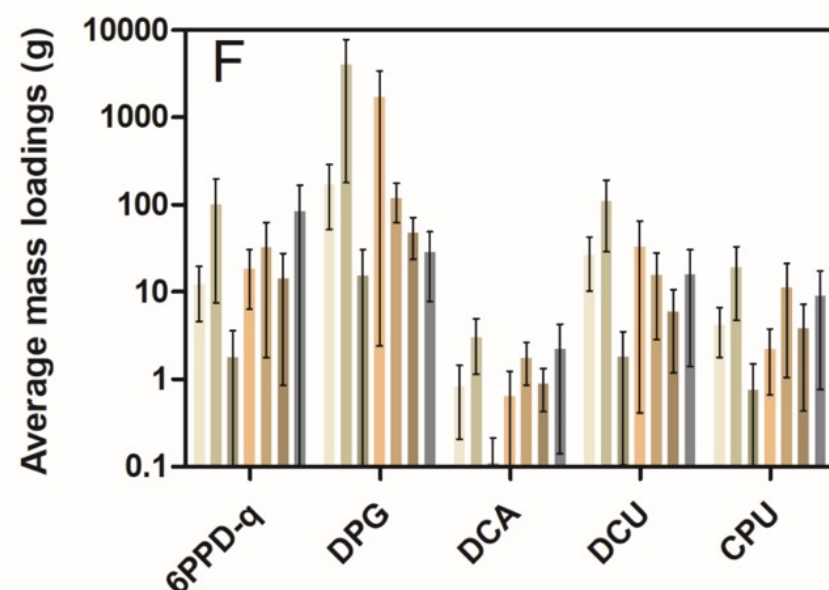
- June 20th sampled approximately 9 hours into storm event (≈ 7 of 25 mm cumulative precipitation)
 - ✓ Peak 6PPD-q concentrations
 - ✓ Average DPG concentrations
- July 24-25 sampled approximately 24-hours into storm event
 - ✓ Mostly non-detection of 6PPD-q
 - ✓ Peak DPG concentrations
- Lag time of DPG? Extended periods of peak concentrations?
 - ✓ Sources, fate, timing of sampling, intensity of rainfall, antecedent dry days, street cleaning...



Stormwater loadings

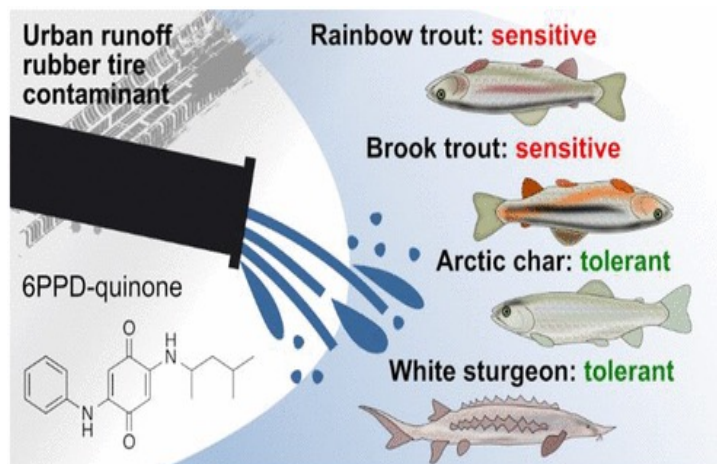


Land-use class	6PPD-quinone
Residential	0.916
Roads	0.878
Commercial	0.666
Industrial	0.389
Green	0.347



- Average mass loads of 6PPD-quinone, DCA, DCU, and CPU ranged from 1-100 g
- DPG mass loadings at multiple sites exceeded 1kg, reaching 16 kg maximally

Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance



Test Species

- Four species of commercial, recreational and aboriginal (CRA) were selected, focusing on salmonids
 - ✓ Rainbow trout (*Oncorhynchus mykiss*) – Economically important species that represents a standard model for ecotoxicity testing
 - ✓ Brook trout (*Salvelinus fontinalis*) – Important sport fish species
 - ✓ Arctic char (*Salvelinus alpinus*) - First Nations sustenance fisheries; Sport fish; Commercial fisheries
 - ✓ White sturgeon (*Acipenser transmontanus*) – Ancient fish endangered in most of its Canadian reaches



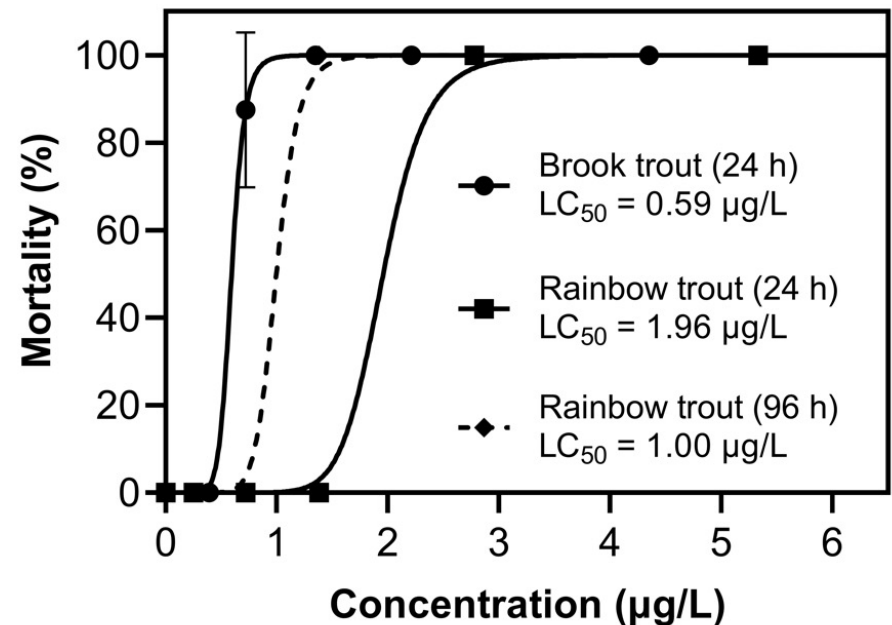
Experimental Design

- 12-96h acute toxicity tests
- Sub-adult life stages (1-4 years of age depending on species)
- Static renewal (~70% water change every 24 hrs)
- Test concentrations informed by limited pilot studies with single replicate high concentration design
- Species-specific concentration ranges
 - ✓ Rainbow trout: 0, 0.15, 0.75, 1.5, 3 and 6 $\mu\text{g/L}$
 - ✓ Brook trout: 0, 0.1, 0.5, 1, 2 and 4 $\mu\text{g/L}$
 - ✓ Arctic char: 0, 20 $\mu\text{g/L}$
 - ✓ White sturgeon: 0, 20 $\mu\text{g/L}$
- Test concentrations confirmed with LC-Orbitrap HRMS



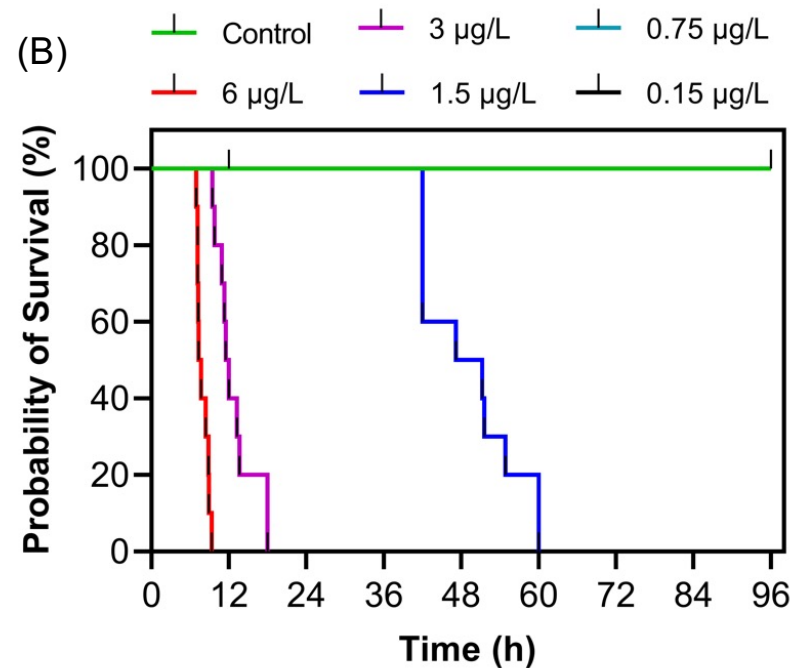
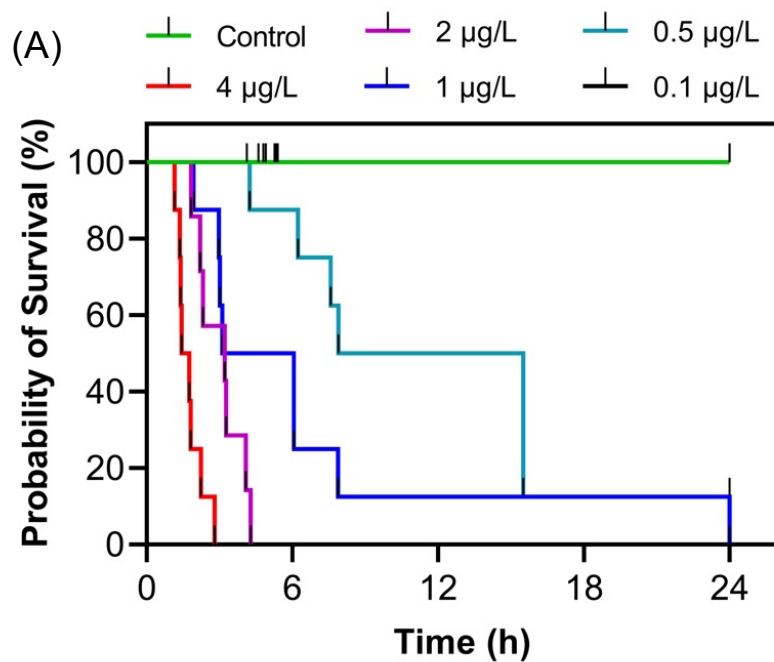
Results

- No mortality occurred for Arctic char and white sturgeon even at the highest concentrations (~13-14 $\mu\text{g/L}$)
- Brook and rainbow trout showed significantly mortality with LC50 values of 0.59 and 1.00 $\mu\text{g/L}$, respectively



Results

- Responses in rainbow (A) and brook (B) trout differed as a function of time with the latter responding much more rapidly than rainbow trout



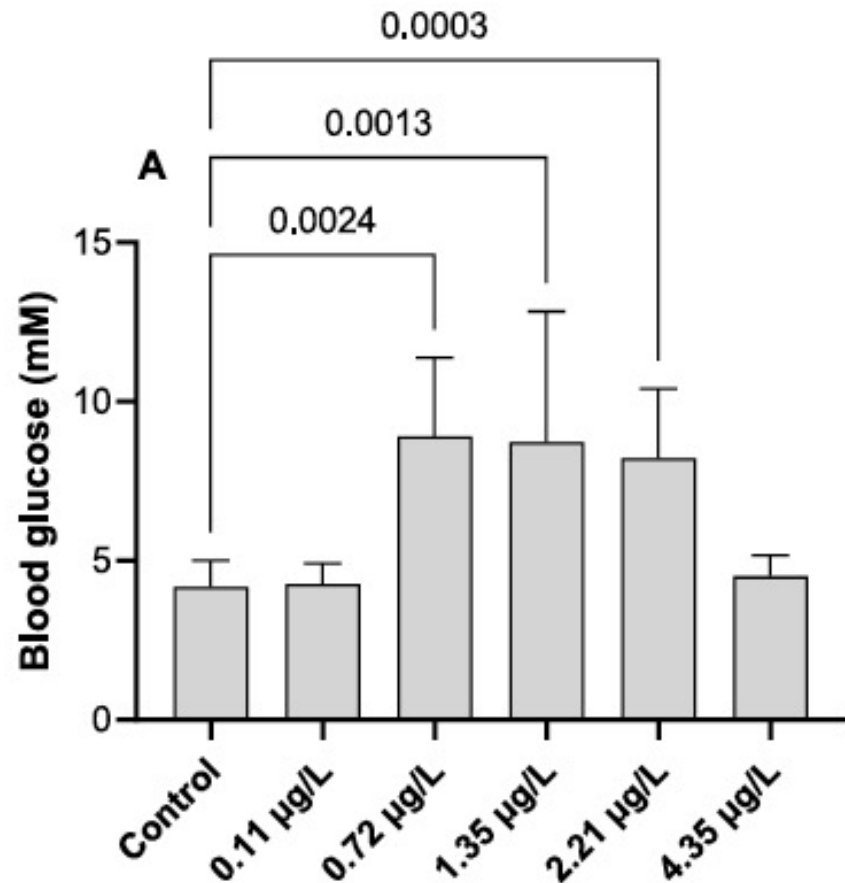
Results

- Unique behavioral patterns prior to fish becoming moribund -> increased ventilation, gasping, spiraling, and loss of equilibrium



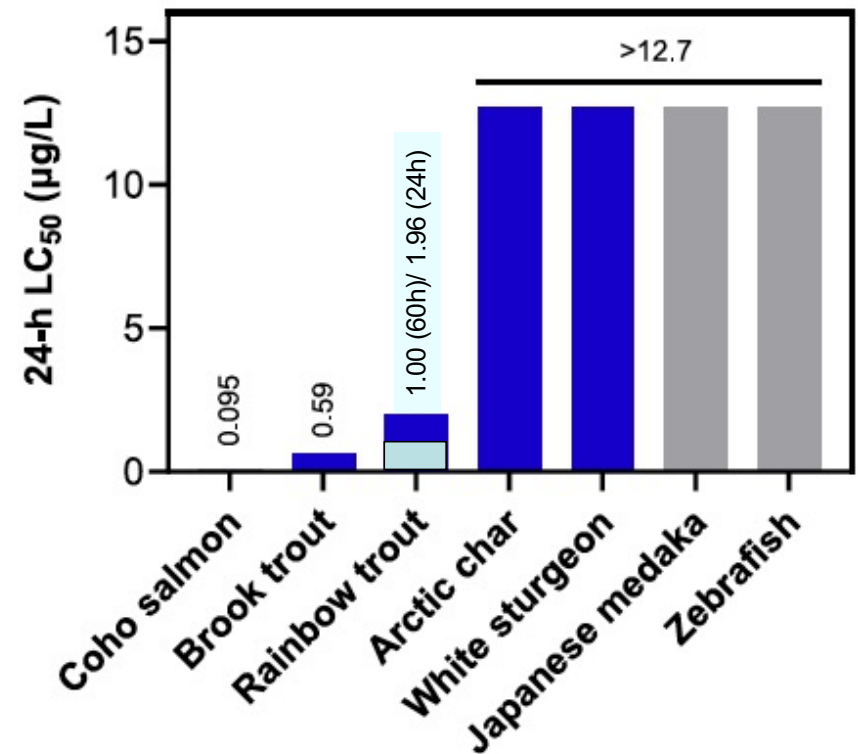
Results/Discussion

- Significant increase of glucose when fish became moribund
- Significant increase in hematocrit
- Suggests that acute exposure to 6PPD-quinone impacted energy metabolism



Discussion

- Significant differences in sensitivity among fishes
- To date salmonids were the only fishes that were sensitive to 6PPD-quinone exposure
- Even species from the same genus showed vastly different responses (*O. mykiss* vs *O. keta* or *S. alpinus* vs *S. fontinalis*)
- Previous and preliminary in-house data suggest invertebrates are insensitive



Conclusions

- Concentrations of 6PPD-quinone measured in urban stormwater runoff agreed well with those reported by other studies
- Concentrations of DPG (>300 µg/L) some of the highest globally
- 6PPD-quinone represents a common contaminant in stormwater that frequently exceeds LC₅₀ for coho salmon, brook trout and rainbow trout

City	6PPD-quinone (µg/L)		Acute LC50 Sens. Species
	Stormwater	Receiving Water	
U.S. Westcoast	0.8-19	<0.2-3.5	0.095-1.00 µg/L
Toronto	0.3-2.3	n.d.	
Saskatoon	0.09-1.4	n.d.	

Conclusions

- Select salmonid species appear to be highly sensitive to acute exposure to 6PPD-quinone (LC₅₀s between 0.095 and 1.00 µg/L)
- There are no clear patterns in sensitivity among fishes, with even closely related species (e.g. from the genus *Salvelinus* or *Oncorhynchus*) showing very different responses
- Acute toxicity for the three sensitive species occurred at concentrations found in stormwater runoff after rain events
- Differences in of response may have significant implications for ecological risk assessment of urban runoff events
- Need to understand the mechanism driving the dramatic differences in sensitivity of fishes to 6PPD-quinone

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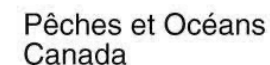
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Thank you!

Questions?